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The Effects of Cell Compressibility, Motility and Contact Inhibition on the Growth of Tumor Cell Clusters using the Cellular Potts Model

Jonathan F. Li^{1,2} and John Lowengrub^{1,*}

¹Department of Mathematics, University of California at Irvine, USA

²Harvard University at Cambridge, USA

Abstract

There are numerous biological examples where genes associated with migratory ability of cells also confer the cells with an increased fitness even though these genes may not have any known effect on the cell mitosis rates. Here, we provide insight into these observations by analyzing the effects of cell migration, compression, and contact inhibition on the growth of tumor cell clusters using the Cellular Potts Model (CPM) in a monolayer geometry. This is a follow-up of a previous study (Thalhauser et al., *Biol. Direct*, 2010, 5:21) in which a Moran-type model was used to study the interaction of cell proliferation, migratory potential and death on the emergence of invasive phenotypes. Here, we extend the study to include the effects of cell size and shape. In particular, we investigate the interplay between cell motility and compressibility within the CPM and find that the CPM predicts that increased cell motility leads to smaller cells. This is an artifact in the CPM. An analysis of the CPM reveals an explicit inverse-relationship between the cell stiffness and motility parameters. We use this relationship to compensate for motility-induced changes in cell size in the CPM so that in the corrected CPM, cell size is independent of the cell motility. We find that subject to comparable levels of compression, clusters of motile cells grow faster than clusters of less motile cells, in qualitative agreement with biological observations and our previous study. Increasing compression tends to reduce growth rates. Contact inhibition penalizes clumped cells by halting their growth and gives motile cells an even greater advantage. Finally, our model predicts cell size distributions that are consistent with those observed in clusters of neuroblastoma cells cultured in low and high density conditions.

Keywords

Tumor growth; cell-cell interactions; cell fitness; computational method; Cellular Potts Model

1. Introduction

Tumorigenesis is a complex process in which cells acquire phenotypes that give them a competitive advantage (e.g., increased fitness) over untransformed cells. Most notably, these phenotypes include unrestricted proliferation and invasive potential, among other cancer

hallmarks [38]. Tumor cells may acquire a high proliferation potential through alterations in the growth signaling pathway. For instance, mutations which permanently activate the growth-regulating Ras gene are found in about 20% of all human tumors [22]. A loss of function of the tumor suppressor gene p53, which is responsible for repairing DNA, regulating the cell cycle, and activating apoptosis, is also implicated in many tumors [47]. Such genetic mutations afford tumor cells a competitive advantage over surrounding tissues.

At the same time, genes associated with the migratory ability of cells have been found to increase cell fitness even though these genes do not directly affect cell mitosis rates. For example, while the cytoskeletal effector RhoC is not an oncogene (since RhoC does not transform normal cells), RhoC does influence cell migration and tumorigenicity [19, 70]. Similarly, overexpression of the cytoskeletal adaptor molecule ABI-1 in breast cancer cell lines enhances cell migration and invasion potential [46], as does the integrin-associated focal adhesion kinase [14, 48].

We wish to understand what combinations of phenotypes give tumor cells enhanced fitness over untransformed cells. In this study, we focus on the individual and combined effects of cell motility, compression, and contact inhibition on tumor growth. In general, cells may move randomly, may move up gradients of chemical fields (chemotaxis), and may migrate along adhesion gradients (haptotaxis). At the sub-cellular level, polymerization and depolymerization of actin-derived cytoskeletal structures are most responsible for facilitating movement [5]. Compressive stress, resulting from proliferation of clusters of tumor cells in a confined geometry, may induce cytoskeletal modifications and the emergence of invasive phenotypes [12, 61, 74]. Motile tumor cells, which are thought to be precursors of metastatic activity [30], tend to be difficult to image and detect and are also more difficult to treat.

We also investigate contact inhibition, a phenomenon that restricts cell proliferation and motility of cells that are in contact with other cells [1]. The process of contact inhibition is well understood. Adherens junctions, or interactions between cells via receptors, play a significant role in signaling a cell to stop growth and movement. The transmembrane receptors nectins, cadherins, integrins and growth factor receptors have all been implicated in the signaling pathway of contact inhibition [68, 72]. A distinctive characteristic of cancer cells is partial or complete insensitivity to contact inhibition [29, 54] although some immortalized cancer cell lines remain sensitive to contact inhibition (e.g., neuroblastoma [13]). In this work, we explore how responsiveness to contact inhibition affects cluster growth and demonstrate the competitive advantage a loss of contact inhibition affords to tumor clusters.

To investigate the tumor behavior, we use a mathematical/computational model. Tumor models can be organized into three broad categories describing simulation dynamics: discrete, continuous, and hybrid. Each type has their benefits and drawbacks, with increased biological detail being associated with increased computational cost. See for example the reviews [7, 15, 20, 28, 50, 60]. Discrete simulations most easily incorporate biological laws and model cellular interactions, but this level of detail demands substantial computational power. Continuous models are useful for simulating the growth dynamics at larger (tissue) scales, but are not able to distinguish among individual cells. Hybrid models [10, 20, 27, 45, 69], which combine features of both discrete and continuous models, are a promising approach but require more development before they can be broadly used.

In a recent study, Thalhauser et al. (2010) investigated the interactions of proliferation, migratory potential and cell death on the emergence of invasive phenotypes using spatial generalizations of the discrete, stochastic Moran process where the size and shape of the

cells is not considered. In this study, migration is found to have a direct positive impact on the ability of a single mutant cell to invade a pre-existing colony and that a decrease in the proliferation potential can be compensated by an increase in the potential for cells to move. Here, we extend the study to include the effects of cell size and shape and cell-cell interactions such as adhesion and contact inhibition.

In this work, we focus on the growth of small cell clusters and we use the Glazier-Graner Hogeweg model (GGH), a discrete lattice-based modeling environment [17, 18, 35, 42, 66, 71]. This model, also known as the Cellular Potts Model (CPM), is very versatile and has been used to model, for example, gastrulation [49, 76], angiogenesis [4, 53, 57, 67], cell sorting [78], somite formation [41], intracellular dynamics [6], vasculogenesis [52, 65] and tumor growth [6, 9, 21, 33, 62, 75]. Continuum limits of the CPM have also been analyzed in the context of cell chemotaxis and solutions are well-described by Fokker-Planck equations for the cell probability density function [2, 3]. Very recently, the CPM has been extended to simulate individual cells migrating in fibrous extracellular matrix [63, 64]. Further, as part of a recent study of cell differentiation and migration, a thermodynamic analysis of the CPM was performed [39]. In [77], an analysis of the CPM at multiple time scales was conducted.

Through our investigation, we find that there is an interplay between cell motility and compressibility within the CPM. Interplay between cell characteristics and interactions has previously been noted as an important aspect of the CPM that requires further study [77]. In particular, the CPM predicts that increased cell motility leads to smaller cells. In fact, for sufficiently large cell motilities, cells may become unstable and actually vanish. This is an artifact in the CPM and we are not aware of any correlations between cell size and cell motility being observed experimentally. An analysis of the CPM enables us to derive an inverse-relationship between the cell stiffness and motility parameters that we use to compensate for motility-induced changes in cell size in the CPM so that in the corrected CPM, cell size is independent of the cell motility.

The remainder of the paper is organized as follows. In Sec 2, we present the CPM model. In Sec 3, results are presented and analyzed. Finally, in Sec 4, we draw conclusions and discuss further work.

2. The Model

2.1. The Cellular Potts Model

The CPM utilizes generalized cells mapped out on a uniform 2D-Cartesian grid. These generalized cells are specially-extended objects composed of a collection of grid cells (sometimes referred to as pixels) and can include both cells and extra-cellular matrix (referred to as “medium”). Each generalized cell is assigned an index, σ , and has cell type τ (σ). Here, we use two different generalized cell types: $\tau=1$ (medium or ECM) and 2 (cell).

The simulation is stochastic and proceeds via a Monte Carlo method by repeating a basic operation, called an index-copy attempt. A grid cell, i , is chosen at random from the simulation grid and attempts to copy its index $\sigma(i)$ onto a neighboring grid cell, i_{neighbor} . The simulation accepts this index-copy attempt with probability given by the Boltzmann acceptance function:

$$P(\text{copy}) = \begin{cases} 1 & \Delta H \leq 0, \\ e^{-\Delta H/T_m} & \Delta H > 0. \end{cases}$$

Here, ΔH is the difference in free energies of the proposed and initial configurations of the entire system. This difference in energy reflects the work done by forces acting by and upon cells [39]. The parameter T_m mimics the temperature and directly affects the probability of a successful copy attempt, e.g., a large T_m increases the rate of successful index-copy attempts. When the index-copy operation is repeated as many times as there are grid cells in the simulation grid, the simulation clock increments by one Monte Carlo step (MCS) and the process is repeated. Here, we present a brief description of the CPM and we refer the reader to references [18, 35, 42, 63, 71] for additional details.

The free energy in our model includes energies associated with cell-cell adhesion, volume constraints and motility:

$$H_{\text{total}} = H_{\text{adhesion}} + H_{\text{volume}} + H_{\text{motility}}. \quad (2.1)$$

The descriptions of the individual terms are included in the next subsections, followed by an explanation of other rules governing the simulation.

2.1.1. Adhesion—In the CPM, the adhesion energy between generalized cells is modeled as

$$H_{\text{adhesion}} = \sum_{\vec{i}, \vec{j}} J_{\tau(\sigma(\vec{i})), \tau(\sigma(\vec{j}))} (1 - \delta_{\sigma(\vec{i}), \sigma(\vec{j})}) \quad (2.2)$$

where J is an interaction energy and δ is the Kronecker delta function. In the simulation consider the case that medium-medium (1,1) and tumor-tumor (2,2) interactions have the lowest energies while medium-tumor (1,2) or (2,1) interactions have the highest energy. Thus, medium-tumor interfaces have high relative energy and their length tends to be minimized. Here, we take $J_{1,1} = 0$, $J_{1,2} = J_{2,1} = 12$, $J_{2,2} = 8$.

The results primarily depend on the ratio of these energies to one another; the actual magnitude of these energies can in principle be related to the strength of cell-cell and cell-matrix receptor binding forces. If the coefficients are scaled upwards while keeping the ratio the same, the adhesion component of the Hamiltonian is weighted more heavily than the volume and motility energies. As we will discuss in Sec. 3.1, an interplay exists between all components of the energy, and changing the weight of one component requires the recalibration of the other two. Thus, if we scale the adhesion energy, we must scale the volume and motility energies, resulting in the total Hamiltonian, H_{total} , being scaled as well. If we refer back to the Boltzmann acceptance function, scaling H_{total} up by certain factor is equivalent to scaling the temperature coefficient, T_m , down by that same factor. Our simulations are robust to temperature variation, with no significant effect on cluster growth when T_m is changed from 4 to 100 (data not shown), although higher temperatures can potentially cause significant cell membrane fluctuations [35]. Thus, while adhesion certainly has a quantitative effect on the results, it does not affect qualitatively the results presented here provided all the energy components are rescaled. Consequently, since we are primarily interested in the interplay between cell motility and compressibility we do not vary the interaction coefficients in the adhesion energy in our studies here.

2.1.2. Volume—In the CPM, deviations of the volume v_σ of a cell σ from a desired cell volume $V_T(\sigma, t)$ are penalized using the energy:

$$H_{\text{volume}} = \sum_{\sigma} \lambda_V (v_{\sigma} - V_T(\sigma, t))^2, \quad (2.3)$$

where λ_V is the cell stiffness (inverse compressibility). In the simulation V_T varies for each cell depending what phase of the cell cycle the cell is in (see Sec. 2.2.1). Changes in volume energy can also be thought of as a measure of pressure a cell is experiencing from its surroundings [39].

2.1.3. Motility—Cell migration is modeled using an ExternalPotential plugin in CompuCell3D [71] to achieve random motion. Each cell is assigned a different random unit vector $\vec{m}$ that determines the direction of movement of the cell. In particular, we take $\vec{m} \equiv (\sin \theta, \cos \theta)$, where θ is a uniformly distributed random variable in the interval $[0, 2\pi)$. The energy associated with cell motility is modeled as

$$\Delta H_{\text{motility}} = \sum_{\sigma} \lambda_M \vec{m}(\sigma, t) \cdot \vec{s}, \quad (2.4)$$

where $\vec{s}$ is the spin flip direction, which is the vector pointing from the current grid cell i to the neighboring grid cell i_{neighbor} where the index copy is attempted. Every 50 MCS, $m(\vec{\sigma})$, a new θ is chosen and the vector $m(\vec{\sigma})$ is updated. Note that this approach does not directly control cell movement. Rather, by altering λ_M , the probability of accepting index-copy attempts is changed, which in turn affects the average speed as well as direction of cells. Changes in the motility energy can be interpreted as the work done by forces exerted by a motile cell [39]. We have checked that this approach models cell motion via a random walk process. In particular, we observe that the mean squared displacement of a single cell increases linearly in time, with the rate of increase (the diffusivity) depending on λ_M , T_m , and the time interval for choosing θ . Increasing any of these parameters increases the effective diffusivity of the cell. In the Supplementary Material, we present characteristic results and effective cell diffusion coefficients obtained by changing λ_M .

Our approach mirrors that for chemotaxis in the CPM where the change in energy has been modeled as (e.g., [71]) $\Delta H_{\text{chemotaxis}} = \sum_{\sigma} \lambda (c(\vec{i}_{\text{neighbor}}) - c(\vec{i}))$, where c is the concentration of the chemical field. The coefficient λ is analogous to λ_M in Eq. (2.4). Both approaches function by biasing movement in certain directions via index-copy attempts.

2.2. Other rules governing cell behavior

2.2.1. Cell Cycle—Many models utilize a two-phase cell cycle: mitosis, the physical process of cell division, and interphase, the period between mitosis where cells double in volume [31, 32, 58]. Others are a bit more elaborate, with the cycle responding to external factors such as nutrient supply and available space [25, 59, 75] or an internal clock [43]. The cells in our model respond to both external and internal cues for progression through the cell cycle.

We focus on the four phases of the cell cycle that affect the volume of the cell: the G1, S, G2, and M phases. We do not model the quiescent phase G0. In the two gap phases, G1 and G2, cells increase their volume by producing macromolecules and organelles, preparing the cell for DNA replication and mitosis. This is modeled by increasing the target volume V_T in time during these phases of the cycle. In the synthesis phase (S-phase), all cell growth is halted temporarily to simulate the stage of DNA synthesis. In the mitosis phase (M-phase), the cell, usually around 50 grid cells, divides into two daughter cells of equal volume along an randomly aligned axis.

In our model, procession from one stage to the next is regulated by both the cell volume v_σ and a timer. We divide a complete cycle into 16 equally-timed stages, with the G1 and S phases persisting for 6 stages each and 4 stages total for G2 and M phases [43]. We scale these times to the model so that the G1, S, G2, and M phases last on average 94, 94, 37, and 25 MCS respectively, taking into account that the M phase is about a tenth of the cycle length. We also account for randomness in the cell cycle as follows. We take the length of each phase to be chosen from a uniform distribution bounded by a 25% deviation from the mean phase length. We note that this variation does not affect the results qualitatively. Also, in order for a cell in the simulation to pass from the G1 to S phase, we place a volumetric restriction, $v_\sigma = 40$, reflecting the effect of mechanical feedback (e.g., contact inhibition—see below) and compression on cell growth. Once the cell has passed the G1 phase, the cell is committed to mitosis and the rest of the phases follow based on the timing described above.

2.2.2. Contact Inhibition—Contact inhibition is a process by which cell growth and movement are halted due to contact with neighboring cells. Contact inhibition is exhibited by healthy tissue to prevent overgrowth, while a hallmark of tumor cells is the loss of contact inhibition. One approach is to model contact inhibition by mechanical stress [23, 32]. Another approach is a contact-dependent growth inhibition, meant to reflect signals cells receive through receptor proteins on the cell membrane. Our simulation implements both approaches. We model the stress effect by imposing a threshold minimum volume in order for a cell to proliferate (see the previous subsection). For contact-dependent growth inhibition, we posit that the more surface a cell shares with another, the less likely it is to grow. We aim to introduce contact inhibition in the simulation to model normal cell growth and compare that to the growth of tumor clusters.

We define the ratio r_s to measure how much of the cell is in contact with other cells:

$$r_s = \frac{s_{\sigma \text{ touching medium}}}{s_\sigma}, \quad (2.5)$$

where s_σ is the surface area of cell σ . The numerator signifies the area of cell membrane touching the medium and reflects the amount of space the cell has to move or grow. Thus, r_s measures the percentage of the cell that is not surrounded by other cells. When $r_s = 1$, this describes the situation of a single isolated cell while a cell with $r_s = 0$ is completely surrounded by other cells. In other words, the parameter r_s is a measurement of space availability.

In the model, if r_s is less than a threshold parameter C_1^* , then cell growth and motility are both arrested by holding V_T constant and setting λ_M equal to 0. However, if $r_s \geq C_1^*$, we follow the procedures described above for determining the cell volume and motility. The parameter C_1^* controls the influence of contact inhibition such that when $C_1^* = 0$, the cells do not respond directly to contact with other cells.

2.3. Physical units and simulation set-up

A single cell in the simulation has a volume (area) of about $v_\sigma = 40$ grid cells and cancer cells are about 20 μm in diameter. If these cells are circular in shape, the width of a grid cell would be about 2.8 μm . We can determine a temporal conversion by assuming that cells divide every 12 h [62]. In the simulation, a complete cell cycle is 250 MCS so 1 MCS is approximately 3 mins.

In our simulation, cells with $\lambda_M = 100$ travel at approximately $0.408 \mu\text{m}/\text{min}$ which is comparable to $0.326 \mu\text{m}/\text{min}$ recorded for cells on a collagen gel [37]. An isolated cell in the simulation undergoes a random walk, in which the direction of movement is changed at periodic intervals. The random variable of cell displacement after a given time interval is given by the probability distribution of a diffusion model. This model predicts that the mean square displacement $\langle r^2 \rangle$ of the cell is proportional to the time interval. In other words, $\langle r^2 \rangle \sim Dt$, where D is the diffusion constant and t is the time elapsed. Indeed, we have verified that this relation holds in our simulations and have estimated the effective cell diffusion coefficient as a function of λ_M (see Supplementary Material).

Our simulations utilize a 500×500 rectangular grid corresponding to a physical domain roughly $1400 \mu\text{m} \times 1400 \mu\text{m}$ in size. Such a grid can comfortably fit a cluster of 5000 cells. Initially, a single cell with size (area) 30 pixels is placed at the center of the grid. Simulations for each set of parameters were replicated 30 times and the average and standard error bars were calculated to generate the figures. A single simulation usually takes between 10–30 minutes to fill the entire grid on a 2.2 GHz Intel Core i7 Mac processor.

3. Results

3.1. Interplay between motility λ_M and stiffness λ_V on cell size and shape

We find that in the CPM, cell size depends on both λ_M and on λ_V . In particular, define the cell compression $C(\lambda_V, \lambda_M)$ to denote the average compression a single cell experiences in the absence of all other factors except for motility and stiffness. More precisely,

$$C(\lambda_V, \lambda_M) = \lim_{MCS \rightarrow \infty} \frac{1}{MCS} \sum_{MCS=1}^{\infty} c_{\sigma}(MCS), \quad (3.1)$$

where

$$c_{\sigma}(t) = v_{\sigma}(t) / V_T(t) \quad (3.2)$$

is the compression of a single cell σ with motility λ_M and stiffness λ_V .

Table 2 presents a set of C -values generated by placing a single cell on a grid with periodic boundaries over the range $1 \leq \lambda_V \leq 10$ and $0 \leq \lambda_M \leq 200$. In order to isolate the effects of λ_V and λ_M , the cell is prohibited from proliferating as to not include cell-cell interactions. The C -value of the cell is recorded at every MCS for 10 runs of 10,000 MCS each. The mean for each run is calculated and is used to determine the average values of C and the standard deviation.

A 3D spline interpolant is used to predict values between the numbers obtained in Table 2. The corresponding plot of cell C -values is shown in Fig. 1. As expected, a large stiffness coefficient λ_V correlates with a C -value close to 1, as in this case cells tend to follow their target volumes closely. On the other hand, increasing the motility coefficient λ_M effectively decreases the C -values of cells. Some values for $\lambda_V = 1$ are not available at larger λ_M as the simulation becomes unstable and the cell often disappears because the volume constraint is so lax that its volume reaches 0.

An examination of Eq. (2.1) explains the interplay between motility λ_M and stiffness λ_V . Consider index copy attempts for which deviations between the actual and desired cell size would prevent the copy attempt from occurring. When the motility parameter λ_M is increased, the motility component of the energy becomes increasingly negative when s^- points in the opposite direction as m . Thus, for sufficiently large λ_M , ΔH may become

negative and the probability of the copy attempt can become equal to 1 even if the actual and desired cell volumes differ greatly. This is an artifact of the CPM as we are not aware of any correlations between cell size and cell motility being observed experimentally.

Using the C -values obtained in Table 2, we are able to determine a relationship between C and the parameters λ_V and λ_M . The data from Table 2 is plotted in Fig. 2 using log-log scale, which suggests an equation of the form:

$$C(\lambda_V, \lambda_M) = 1 - \frac{b(\lambda_M)}{\lambda_V^{p(\lambda_M)}}, \quad (3.3)$$

where the values of the coefficient b and the exponent p are given in Table 3 for different motility coefficients λ_M . While there is some variability in b , the power p is quite close to 1. This inverse relationship between C and λ_V is reasonable because it suggests that λ_V should increase with λ_M in order to maintain the targeted cell size. This is consistent with the energy formulation since the energy contributions from motility and volume scale together. Equation (3.3) neatly reflects the results shown in Table 2 and so Equation (3.3) is used to calculate all further C -values taking values of p and b from data from Table 3. In particular, as described below, we consider cell cluster evolution using pairs of parameters (λ_V, λ_M) such that average compression C remains the same.

At this point, we also check the shape of the cell as a function of λ_V and λ_M . We define the shape factor $R_\sigma = k \cdot v_\sigma / s_\sigma^2$ to quantify the shape of cell σ (keeping in mind that in the 2-dimensional field we have defined, the volume v_σ , refers to the number of pixels in a cell, and surface area s_σ refers to the cell perimeter). We have normalized the ratio so that the maximum possible value of R_σ is 1. This occurs when the cell takes on a square shape, leading to largest area for a given perimeter (in continuous space, a large R_σ would describe a circular cell). In this case, a square of side s would mean $v_\sigma / s_\sigma^2 = 1/16$, so we normalize all values by dividing by $1/16$ (i.e. $k = 16$). A small value of R_σ describes a cell with high surface area to volume ratio such as disk-like cells or cells with fingered boundaries. On the other hand, a large R_σ close to 1 describes a square-shaped cell. To study morphology, we simulate the behavior of a single, non-proliferating cell. Increasing the stiffness parameter λ_V causes v_σ and s_σ to rise, as one would expect since the cell is encouraged to be larger in size. However, while volume and surface area increase with stiffness, R_σ does not show any significant variation, as shown in Table 4. This implies that altering the stiffness parameter affects the size of cells but does not seem to influence their shape. However, when we increase cell motility λ_M , R_σ decreases, indicating a more elongated or figured cell shape for higher motilities.

3.2. The growth of cell clusters

3.2.1. The effect of cell compression—We next assess the effect of cell compressibility on the growth of cell clusters. We first do not factor in motility, setting the motility coefficient $\lambda_M = 0$. We also do not yet consider contact inhibition in this simulation and allow unimpeded growth in the G1 and G2 phases. Fig. 3 shows the volumes of the tumor cell clusters for two different cell stiffnesses, $\lambda_V = 2$ and 5, with solid curves denoting the mean and the bars showing the standard deviation for results obtained by averaging over 10 runs. We see that cells with large λ_V grow more quickly than those with smaller λ_V . This faster growth is a result of a difference in both cell volume and number. Compression in the center of the tumor induces a deviation between their actual volumes and target volumes of the cells. In experiments, compressive stress, resulting from proliferation of clusters of tumor cells in a confined geometry, often results in cytoskeletal modifications and the emergence of invasive phenotypes [12, 61, 74]. In our simulations, cells with larger λ_V are

more resistant to compression and are more likely to follow their growing target volumes. Our simulations show that, in addition to having a larger average cell size, tumors containing cells with large λ_V also contain more cells since cells with larger λ_V are better able to resist compression and therefore can undergo mitosis more readily than cells with smaller λ_V .

Fig. 4 illustrates typical compression patterns for tumor clusters with $\lambda_V=2$ and 5, where the cells are color-coded (see online version of the paper) based on the individual cell compression c_σ defined earlier in Eq. (3.2). In each image, cells in the center tend to be compressed while cells near the tumor boundary are less compressed. This result is consistent with previous findings [16, 24, 31, 32, 51] which show that cells on the border of the monolayer experience much less pressure than those in the center. And, because the cells on the border experience less compression, their volumes tend to be larger. As the tumor increases in size at late times, cells on the tumor boundary hit the boundary of the computational domain and the simulation is stopped. Cells in contact with the computational boundary also show increased compression.

The distributions of the corresponding individual cell volumes are shown in Figs. 5(A) and (B) for $\lambda_V = 2$ and $\lambda_V = 5$, respectively. Comparing the two graphs, observe that the distribution for $\lambda_V=5$ is shifted slightly towards larger volumes and is narrower than that for $\lambda_V=2$. This is consistent with cells with larger λ_V being more resistant to compression. Indeed, comparing the compression ratios c_σ for the two cases (Figs. 5(C) and (D)) clearly demonstrates that the cells with $\lambda_V=5$ are less compressed and follow the target volume more closely. In addition, we notice that there are two peaks in both the cell volume histograms (Figs. 5(A) and (B)), although the peaks are more pronounced for $\lambda_V = 5$. These peaks describe two distinct cell populations in a tumor cluster arising from different levels of compression experienced in the center and on the perimeter of the cluster. Bimodal distributions have also been observed in neuroblastoma cell culture experiments (D. Stupack, personal communication).

3.2.2. The effect of cell motility—Next we analyze the effect of cell motility on cluster growth. Table 5 shows how the initial growth rate α (e.g., $\dot{X} \sim \alpha X$, where X is the total volume of the cluster) changes with stiffness λ_V and motility λ_M . We would expect from previous modeling work [73] that clusters containing highly motile cell lines would grow quickly since the motile cells are more diffusive and thus, less competitive for space to grow. Less motile cells are more likely to be in contact with one another and experience compression, whereas more freely moving cells are more likely to be in contact with medium (ECM) and to be able to expand close to their target volume.

Considering the individual columns in Table 5, we notice that λ_V correlates with faster growth, corroborating the results achieved in the previous section. The table also shows that increasing λ_M from 0 to 50 while keeping λ_V constant does not always lead to an increased initial growth rate, in contrast to the results obtained by Thalhauser et al. This is due to motility-induced cell compression as discussed earlier in Sec. 3.1.

Indeed, a comparison of simulations in which the C -values in Eq. (3.1) are fixed supports our hypothesis. Since $C(\lambda_V, \lambda_M) = C(2, 0) = C(3.427, 100) = C(4.314, 200) = 0.93$, cells in simulations with these parameters will experience the same overall compression. Once the motility-induced size changes of cells are corrected in the CPM, the remaining cell compression (e.g., changes in cell volume due to cell-cell interactions) is associated with the generation of solid pressure [39]. The corresponding initial growth rates α can be extrapolated from the entries in Table 5. We find that under these conditions, clusters containing motile cells have consistently larger initial growth rates as λ_M is increased, in

agreement with previous work in which cell compressibility and surface contact inhibition were neglected [11, 16, 26, 40, 73]. This is further confirmed in Table 6 where the results obtained by fixing average compression values at $C(2, 0)$, $C(3, 0)$ and $C(4, 0)$ are shown for $\lambda_M = 0, 50, 100$ and 200 .

We study the compression patterns for these simulations and plot the results in Fig. 6. As expected, the darker, more compressed cells are generally in the centers of the tumors while the lighter, less compressed cells are on the periphery. These peripheral cells are larger and have more irregular shapes. The cluster of cells with $(\lambda_V, \lambda_M) = (2, 200)$ in Fig. 6(B) is much smaller and the cells are more compressed than the other two cases since $C(2, 200) = 0.827 < C(2, 0) = C(4.314, 200) = 0.93$. Interestingly, the cluster with $(\lambda_V, \lambda_M) = (4.314, 200)$ in Fig. 6(C) contains more compressed cells than the cluster with $(\lambda_V, \lambda_M) = (2, 0)$ shown in Fig. 6(A). This is because the increased motility results in a larger cluster, which grows against the boundary of the computational domain. Note that the cluster itself is more irregularly shaped than that with $\lambda_M = 0$.

The corresponding cell volume distributions are shown in Fig. 7. When $(\lambda_V, \lambda_M) = (2, 200)$ in Fig. 7(B), the cells have smaller sizes and the distribution is broader than those shown in Figs. 7(A) and (C), which correspond to $(\lambda_V, \lambda_M) = (2, 0)$ and $(\lambda_V, \lambda_M) = (4.314, 200)$ respectively. Observe that the distribution in (C) is narrower than that in (A) even though the majority of cells in (C) are smaller than those in (A).

3.2.3. The effect of contact inhibition—Finally, we assess the effect of contact inhibition on cell cluster growth. Figs. 8(A)–(C) show results for constant compression ($C = 0.930$) while the contact inhibition threshold and motilities are varied ($C_I^* = \{0, 0.05, 0.2\}$ and $\lambda_M = \{0, 100, 200\}$). In all three figures, the larger compression threshold (e.g., stronger contact inhibition) correlates with slower cluster growth. A more restrictive contact inhibition parameter C_I^* amplifies the effect that cell motility has on cluster growth rates. Cells in the center of the cluster are completely surrounded so their free-surface parameter is less than the threshold ($(r_s < C_I^*)$), e.g., see Fig. 9, which shows cluster morphologies for $C_I^* = 0.2$ with $\lambda_M = 0$ in (A) and $\lambda_M = 200$ in (B). Interior cells experience compression contact inhibition which prohibits them from reaching the $v_\sigma > 40$ threshold for mitosis, as observed in other studies (e.g., [16, 31, 32, 51]). The results also confirm that increasing motility increases cluster growth rates, consistent with Sec. 3.2.2. When $\lambda_M = 0$ in (A), the clusters are compact and the number of cells at the periphery is highly constrained. When $\lambda_M = 200$ in (B), the cluster boundary is much more irregular due to increased cell motility and thus contains a larger number of cells. These boundary cells are then able to find space and proliferate leading to faster growth and enhancement of boundary irregularity. A difference in cluster growth rates is also observed in Figs. 8(A)–(C), with the $\lambda_M = 100, 200$ clusters growing much faster than $\lambda_M = 0$. There is little difference in rates between the $\lambda_M = 100, 200$ cases because the effect of motility on growth is saturated.

This study of contact inhibition is particularly revealing for explaining why tumor clusters are more viable than healthy tissue. Normal tissue cells experience contact inhibition and a hallmark of tumor cells is the loss of contact inhibition, although some immortalized cell lines remain sensitive to contact inhibition (e.g., neuroblastoma [13]). As the simulation data reveals, a complete loss of contact inhibition gives the rate of growth in tumor clusters a significant advantage over normal tissue.

3.3. Cell volume distribution study motivated by in vitro experiments

Unpublished experimental data provided to us by D. Stupack (personal communication) on the growth of in vitro clusters of neuroblastoma cells reveals interesting cell volume

distribution patterns. The in vitro data shows that a bimodal cell volume distribution occurs in cell cultures that were plated in low densities. However, a unimodal cell volume distribution is observed in high density cultures. These observations served as motivation for the simulations described below.

We model the low density case by considering the growth of a single cluster of cells starting from a single initial cell. To model the high density case, cells were seeded close enough to each other such that the ensuing cell clusters strongly interact. The initial configurations and cluster evolutions are shown in Figs. 10(A) and (B), using the parameters: $\lambda_M=0$ and $\lambda_V=2$.

A small contact inhibition threshold $C_1^*=0.05$ is used for both the low (A) and high (B) density cases, which is consistent with results for neuroblastoma [13]. A low motility condition was chosen as higher motility cells did not display the two peaks as observed in the experimental data (this is also consistent with the numerical results presented earlier in Figs. 7 (B) and (C) where the cell size distributions are unimodal when λ_M is large).

The cells in Fig. 10 are colored by compression c_σ . Observe that in the low density case, the cells in the cluster interior are compressed while those on the boundary are less compressed. In the high density case, most of the cells are compressed while only a few on the boundary are less compressed. We plot the corresponding distributions of cell sizes in Figs. 11 (A) and (B). The cell size distributions are bimodal in the low density case (A) and unimodal in the high density case (B), consistent with experimental observations. The bimodal distribution in Fig. 11 appears to be amplified by contact inhibition (data not shown). Indeed, in the contact inhibited case, the central and peripheral cell populations are more distinct as inhibited cells will be stuck in the G1 phase. In the absence of contact inhibition, a bimodal distribution is still observed (e.g., Fig. 5), although the two peaks are not as distinct. Thus, the model predicts that contact inhibition enhances bimodality in the cell volume distribution.

4. Conclusion

In this work, we have used a Cellular-Potts modeling (CPM) approach to study the individual and combined effects of cell compression, motility, and contact inhibition. We explored an interplay between cell motility and compressibility within the CPM and found that increased cell motility leads to smaller cells. We could not find such a relationship in the biological literature and concluded that this is an artifact of the CPM. An analysis of the CPM showed that there is an explicit inverse-relationship between the cell stiffness and motility parameters. We used this relationship to compensate for motility-induced changes in cell size in the CPM so that in the corrected CPM, cell size is independent of the cell motility.

We found that tumor clusters grow larger and contain more cells when the cells are less compressible. These stiffer cells are able to follow their target volumes more closely than their more compressed counterparts, giving them larger volumes and allowing them to replicate more quickly. Interestingly, it has been reported that cancer cells are more compressible than normal cells [36], although normal cells proliferate at much lower rates. Experimentally, it is found that compressive stresses result in changes in cell size and shape, and may lead to the emergence of invasive phenotypes [12, 61, 74]. Further, the responses of normal and tumor cells to such stresses may also be different (e.g., [55]) and may also alter with treatment (e.g., [44]). These features can be incorporated in future models using the approach developed here.

The cluster sizes and cell numbers were also found to be increasing functions of cell motilities, consistent with previous modeling studies that do not consider cell size and shape

(e.g., [8, 11, 25, 40, 73]) and biological observations where high motility rates have been linked to invasiveness, a key property of tumor cells (e.g., [14, 19, 34, 38, 48, 70]). Motility allows cells to find free space where they experience less compression and growth inhibition. Further, we have demonstrated that the effects of motility on cluster sizes were amplified under conditions of contact inhibition. In these situations, the cells can only proliferate, and move, if they have sufficient space, making it even more important for cells to find free space. Cells at the centers of tumor clusters were most affected.

Finally, we conducted a study of the effects of initial seeding densities on cell volume distributions. Our model predicts that when cells are seeded initially at low densities, contact inhibition results in the emergence of two distinct cell populations— a population of larger cells located at the cluster boundary and a population of smaller cells confined to the cluster interior. When the cells are seeded at high densities, most of the cells are compressed and the cluster population is dominated by compressed cells leading to a unimodal cell volume distribution. These results are qualitatively consistent with unpublished data.

As a next step, we plan to work with Dr. Stupack to perform quantitative comparisons of model and experimental results. Further, we plan to investigate cases in which the cells have varying motilities and to study the dynamics in mixtures of such cells, comparing the experimental results with model predictions. We also plan to further investigate the effect of cell shapes and surface areas, which also have the potential to influence the results (e.g., [23, 24, 31, 56, 58]). For example, some cells may naturally have large surface area to volume ratios while other cells may have small ratios. In the CPM, this can be modeled by introducing an additional component to the energy that constrains the cell surface area (e.g., [71]).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
 Compression C -values from Table 2 using a mesh created with cubic spline interpolation.
 Cells have smaller C -values for large λ_M and larger C -values for large λ_V .

Figure 2.

A plot of $\ln(1 - C)$ vs. $\ln(\lambda_V)$ using the data in Table 2 suggests the relationship:

$$C = 1 - b(\lambda_M) / \lambda_V^{p(\lambda_M)} \text{ with } p \approx 1.$$

Figure 3. Growth curves for λ_M and $\lambda_V = 2$ and 5 , where the solid lines correspond to the mean volumes of the cell clusters and error bars denote the standard deviation for 10 realizations of the model.

Figure 4.

Images of tumor cell clusters for $\lambda_V = 2$ (A) and 5 (B), with $\lambda_M = 0$ and $C_I^* = 0$ at $MCS = 2000$ (8 days), 2500 (10 days), 3000 (12 days), and 3500 (14 days). Cells are colored by compression: $c_\sigma = v_\sigma / V_T$.

Figure 5.

Histograms at $MCS = 3500$ (14 days) for (A) cell volume with $\lambda_V = 2$, (B) cell volume with $\lambda_V = 5$, (C) the compression c_σ with $\lambda_V = 2$, (D) c_σ with $\lambda_V = 5$.

Figure 6.

Images of tumor cell clusters with $(\lambda_V, \lambda_M) = (2, 0)$ in (A), $(2, 200)$ in (B), and $(4.314, 200)$ in (C) at $MCS = 2000$ (8 days), 2500 (10 days), 3000 (12 days), and 3500 (14 days).

Figure 7. Histograms of cell volumes from the tumors clusters in Fig. 6 at $MCS = 3500$ (14 days) for $(\lambda_V, \lambda_M) = (2, 0)$ in (A), $(2, 200)$ in (B), and $(4.314, 200)$ in (C).

Figure 8.

Growth curves with $C_I^* = 0, 0.05$, and 0.2 in each graph. In (A), $\lambda_M = 0$, in (B) $\lambda_M = 100$ and in (C) $\lambda_M = 200$. The insets feature the curves for the first 2000 MCS (8 days). The curves denote mean volumes and the error bars denote standard deviations for 30 realizations of the model. In all cases, the compression is fixed at $C(\lambda_V, \lambda_M) = 0.93$.

Figure 9.

Images of tumor cell clusters from Fig. 8 for $C_1^* = 0.2$, $\lambda_V = 2$, and $\lambda_M = 0$ in (A) and 200 in (B) at $MCS = 2000$ (8 days), 2500 (10 days), 3000 (12 days), and 3500 (14 days). Cells are color coded based on the available area ratio r_s .

Figure 10.

Images of tumor cell clusters from the model at MCS=0, 1500 (6 days), and 3000 (12 days) for the cases shown in Fig. 11. (A): Low initial cell density (single cell); (B): High initial cell density (multiple cells spread evenly throughout the domain).

Figure 11. Volume histograms generated by the model simulating (A) low initial cell densities and (B) high initial cell densities. Note the bimodal distribution in (A) and the unimodal distribution in (B). See text for parameters.

Table 1

List of model variables and parameters.

Parameter	Description	Values (if applicable)
$\sigma(i) \rightarrow$	Index of grid cell $i \rightarrow$	
$\tau(\sigma(i)) \rightarrow$	Cell type at grid cell $i \rightarrow$	1 = medium (non-tumor cell); 2 = tumor cell
T_m	Temperature of domain;	4
$J_{\tau(\sigma(i)), \tau(\sigma(j))}$	Adhesion energy	$J_{1,1} = 0, J_{1,2} = J_{2,1} = 12, J_{2,2} = 8$
λ_V	Volume constraint magnitude	2, 5 (Figs. 3–5); 2, 4.3 (Figs. 6,7); 2 (Figs. 8–11)
v_σ	Volume of cell σ	
V_T	Target volume	updated between 25–50
s_σ	Surface area of cell σ	
λ_M	Motility magnitude	0 (Figs. 3–5,10,11); 0, 200 (Figs. 6,7,9); 0, 100, 200 (Figs. 8)
$m \rightarrow$	unit random vector describing the direction of movement	
C_I^*	I Contact Inhibition threshold	0 (Figs. 3–7); 0, 0.05, 0.2 (Figs. 8,9); 0.05 (Figs. 10,11)

Compression C -values, $C(\lambda_V, \lambda_M)$ from Eq. (3.1). $C = 1$ denotes uncompressed cells. Data unavailable for $\lambda_V = 1$, $\lambda_M > 60$ because these cells are not stable and spontaneously vanish.

Table 2

$\lambda_M = 0$	10	20	30	40	50	60	70	80	90	100	200
$\lambda_V = 1.0$	0.853	0.835	0.819	0.812	0.812	0.806	0.795	—	—	—	—
2.0	0.930	0.921	0.916	0.912	0.911	0.909	0.905	0.897	0.890	0.882	0.874
3.0	0.955	0.948	0.945	0.944	0.942	0.941	0.938	0.933	0.929	0.925	0.921
4.0	0.966	0.962	0.960	0.958	0.957	0.956	0.954	0.951	0.948	0.945	0.943
5.0	0.972	0.970	0.968	0.967	0.966	0.965	0.964	0.962	0.959	0.957	0.955
6.0	0.976	0.975	0.974	0.973	0.972	0.971	0.970	0.968	0.967	0.965	0.963
7.0	0.979	0.978	0.977	0.977	0.976	0.975	0.974	0.973	0.972	0.971	0.969
8.0	0.981	0.980	0.980	0.980	0.979	0.978	0.978	0.977	0.976	0.975	0.973
9.0	0.983	0.982	0.982	0.982	0.982	0.981	0.980	0.979	0.978	0.977	0.976
10.0	0.985	0.984	0.984	0.984	0.983	0.983	0.982	0.982	0.981	0.980	0.979

Table 3

The coefficient b and exponent p obtained by fitting the data in Table 2 with the form in Eq. (3.3).

λ_M	Power (p)	Coefficient (b)
0	0.980	0.140
10	1.017	0.161
20	1.051	0.177
30	1.062	0.185
40	1.051	0.186
50	1.051	0.191
60	1.057	0.201
70	1.087	0.223
80	1.106	0.244
90	1.114	0.259
100	1.130	0.280
200	1.160	0.380

Table 4

Scaled values of v_σ/s_σ^2 for varying stiffness λ_V and motility λ_M parameters. All values are normalized by the theoretical maximum v_σ/s_σ^2 .

	$\lambda_M = 0$	10	20	30	40	50	100	200
$\lambda_V = 1$	0.7256	0.7091	0.6762	0.6413	0.6064	0.5683	-	-
2	0.7226	0.7086	0.6736	0.6373	0.6024	0.5722	0.4810	0.4531
3	0.7229	0.7072	0.6734	0.6387	0.6034	0.5629	0.4770	0.4442
4	0.7229	0.7070	0.6738	0.6390	0.6037	0.5720	0.4749	0.4368
5	0.7232	0.7070	0.6742	0.6386	0.6061	0.5747	0.4776	0.4280

Table 5

Initial growth rates (α) for $\lambda_M = 0, 50, 100, 200$ and $\lambda_V = 1, 2, 3, 4, \text{ and } 5$. Data for $\lambda_V = 1$ are missing because cells are not stable for large λ_M and spontaneously vanish.

	$\lambda_M = 0$	$\lambda_M = 50$	$\lambda_M = 100$	$\lambda_M = 200$
$\lambda_V = 1$	0.0011511	0.0011517	–	–
2	0.0011635	0.0011711	0.001169	0.0011771
3	0.0011687	0.0011721	0.0011748	0.0011835
4	0.0011739	0.0011726	0.0011774	0.0011844
5	0.0011874	0.0011794	0.0011795	0.0011887

Table 6

Initial growth rates (α) for $\lambda_M = 0, 50, 100, 200$ when the average compress-ibilities are matched to those values obtained from using $C(\lambda_V, \lambda_M) = C(2, 0), C(3, 0)$; and $C(4, 0)$.

	$\lambda_M = 0$	$\lambda_M = 50$	$\lambda_M = 100$	$\lambda_M = 200$
$C(2,0) = 0.930$	0.0011635	0.0011718	0.0011761	0.0011850
$C(3,0) = 0.955$	0.0011687	0.0011726	0.0011794	0.0011868
$C(4,0) = 0.966$	0.0011739	0.0011793	0.0011833	0.0011875