Using fractal dimension in tumor growth evaluation

RADU DOBRESCU *, CATALIN VASILESCU**, LORETTA ICHIM*
*POLITEHNICA University of Bucharest, Faculty of Control & Computers
** Fundeni Clinical Institute, Department of General Surgery and Liver Transplantation
ROMANIA

Abstract: - This paper proposes a model to simulate the growth of tumors in two dimensions and to investigate the role of a random decrease in the adhesion forces of tumor cells and its effect on tumor morphology. The experimental results conclude that a decrease in cell adhesion, controlled by a model parameter σ, can explain both tumor surface roughening and cell detachment. In the same time it was shown that tumor boundary fractal dimension and roughness increase with σ and consequently that a decrease in adhesion is relevant for the tumor transition from benign to malignant. In this way it was demonstrated that the morphology of the invading front is influenced by changes in the adhesiveness parameters. The simulation was extended by including proliferation and so it was established that an increased proliferation rate usually results in an increased depth of invasion.

Key-Words: Cancer, Tumor morphology, Cell adhesion, Fractal dimension, Computer modeling, Proliferation

1 Introduction

A tumor is an uncontrolled cellular growth initiated by a single cell due to mutations in its genome. Tumor cells do not obey hormone or nervous signals implying that local homeostasis have been lost. All cells in a tumor are clones, i.e., they descend from the same initially mutated cell. During tumor growth and evolution, mutations continue and cells behave differently from the normal cells of the tissue where they appeared. In advanced stages of cancer, cells start to detach from the tumor and invade the blood stream or lymphatic system. There they can be carried to other body parts producing new tumors (metastatic or malignant cancer). The detachment and invasion of other tissues result in part from the incorrect expression of adhesion molecules on the cell's surface for the mutated genome. This process causes a decrease in cellular adhesion between cells with additional consequences such as an increase in mobility of the cells on the surface of the tumor [1]. As a result, the boundaries of the tumor become very irregular. This change on tumor morphology, associated with additional information, help physicians to diagnose cancer stage of development [2]. From about 10 years, starting with the seminal paper of Landini and Rippin [3], several studies indicate that the fractal dimension of tumors is useful as an indicative of malignancy. As a continuity of previous studies [4], [5], [6], in this work the authors propose a solution to investigate tumor roughening based on the fractal dimension of a tumor, obtained both from specific medical images and from computer generated graphical models. The aim is to demonstrate both the validity of the tumor growth model and the accuracy of the fractal dimension discriminator, especially in order to establish the initial transition from the benign to the malignant stage of a tumor.

2 The model of the tumor growth

The characteristics of a differentiated cell are normally tightly controlled by a variety of genetic, local and hormonal controls. When this control is lost and a cell begins to divide excessively, break contact with its neighbors and migrate into the extra-cellular medium, the host is at risk of developing a malignant tumor. Such tumors are aggressive, have a high metabolic rate, can be hormonally active and are able to invade surrounding healthy tissue and spread elsewhere in the body. Typically a finger of cells from the main tumor mass penetrates the basement membrane and begins to invade the surrounding stroma. Invasive cells are less adhesive, more highly mobile, more metabolically active, and more highly mitotic than normal cells. The series of changes leading a normal cell to become malignant and invasive are related to each other and possibly

occur in a stepwise manner, with each mutation following the next. These mutations affect the cell's adhesiveness, its ability to secrete matrix degrading enzymes, its capacity for uncontrolled proliferation, but in addition exhibit changes in their motility. This can be classified into both random movement (chemokinesis) and directed movement along gradients of either diffusible materials (chemotaxis) or fixed substrates (haptotaxis). Due to the dissolution of the extra-cellular matrix and the movement of the cells into it, the cells find themselves in a less dense milieu compared with the interior of the solid tumour. Correspondingly, their random movement is increased. In addition, the reduction or absence of cell–cell contacts by invasive cells may be an independent factor in increasing random movement through a reduction in contact inhibition.

Mathematical modeling provides a means by which we may quantify the various phenomena involved in the invasion process, and investigate their interactions. By using experimental results for the various microscopic quantities involved, we may build up models which reveal the relationships between cellular and biochemical parameters and macroscopic phenomena. Previous modeling techniques for the invasion process have included using sets of coupled reaction–diffusion equations for the cells and important groups of extra-cellular proteins and nutrients [7]. The inclusion of adhesion has proven problematic in this type of model, because the reaction–diffusion approach makes the inclusion of the stochastic behavior of individual cells difficult to treat. In this study, we approach the modeling of invasion in a similar manner as in the work of Turner and Sherratt [8], which have implemented the extended Potts model first proposed by Graner and Glazier [9], but is in this case developed further for application to malignant invasion. The simulation models an individual cell as occupying a defined region of a square lattice. Another difference is that instead to employ a stochastic energy minimization technique to display the evolution of the cell mass over time, the proposed algorithm determines completely the cell motion from the interaction forces between cells and the mitotic rate.

The simulation algorithm model a collection of biological cells by attaching to each lattice point *(i,j)* of a square lattice a label s_{ij}. Adjacent lattice sites with the same value s_{ij} are defined to lie within the same cell. We model the interactions of cell surfaces with each other explicitly by defining coupling constants $J_{ss'}$, the size of which quantifies the strength of the interaction between adjacent lattice points with differing values of s_{ij}. The main features of the model are the following:

1) Cell affinity or adhesion is modeled with decreased repulsion that depends on the cell types.
2) Cell motion results from equilibration of the interaction forces with other cells.
3) The visualization of the cellular tissue is generated computing the Voronoi polygons associated to each particle in the simulation.
4) The simulation starts from an initial tumor cell in a tissue of normal cells.
5) The initial tumor cell and its descendants have a higher division rate than the normal cells and random adhesion parameters obtained from a Gauss-Heaviside distribution.
6) Cell death is not considered.

Cells interact through a force defined as

$$f = (1 - r/r_0)(1-\alpha_{ij}) \quad (1)$$

Each cell has a repulsive core $(1 - r/r_0)$ where r is the distance between the two cells and r_0 is the target cell radius. Cells do not interact if $r > 2r_0$. α_{ij} is a parameter that models adhesion by decreasing the repulsion between cell types i and j. $\alpha_{ij} \sim 1$ imply high adhesion and respectively $\alpha_{ij} \sim 0$ imply low adhesion. The interaction force between normal cells is a function of r only, i.e. they all have the same adhesion parameter α_n. However the adhesion forces of tumor cells are, in general, lower than that of normal cells. This feature is introduced in the simulation by attributing to each tumor cell a random α obtained from a statistical distribution. The distribution function of forces of real tumor cells is unknown. Thus, for simplicity, we assume that the α's of tumor cells follow a Gaussian distributed decrease with standard deviation σ in relation to α_n. This implies that for a constant r in equation (1), the forces of interaction of tumor cells with normal cells have a Gauss-Heaviside distribution $P(f)$ as depicted in fig 1. The average force between tumor cells and normal cells is lower than F_n.

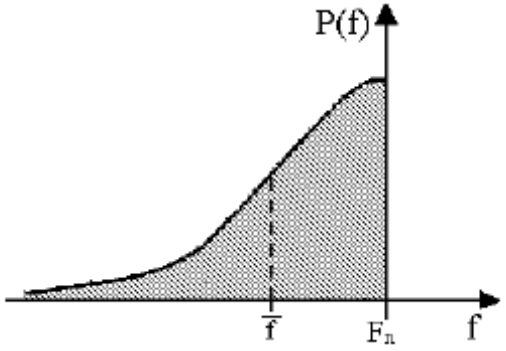


Fig.1 Plot of the Gauss-Heaviside distribution

Interactions involving tumor cells require a rule to determine the force between normal and tumor cells and between tumor cells themselves, since each tumor cell has a random α obtained from the Gauss-Heaviside distribution. We define the value of the adhesion parameter between a normal and a tumor cell as the α of the tumor cell, and between two tumor cells as the geometric mean of the forces of interaction between the two cells.

Simulations were run using a wide range of parameter values, and the model was found robustly to reproduce the phenomenon of ‘‘fingering’’ across a wide range of these values. This phenomenon is illustrated in Fig. 2.

Fig,2 Appearance of a simulated tumor

The surface appearance of many malignancies has this morphology, and it corresponds to disease severity: benign tumors are smooth, whereas aggressive malignancies are ‘‘rough’’. There is a correlation between this ‘‘roughness’’ and the tumor’s invasive potential: studies of photomicrographs of tumor surfaces have succeeded in demonstrating self-similarity at different length scales, and have noticed a relationship between the fractal (Hausdorff) dimension of the tumor surface and its invasive potential

3 Fractal dimension and roughness

To measure the fractal dimension and roughness of the tumor boundary we select only the cells at the boundary of the tumors. We define boundary cells as those that have at least one normal neighbor. In fig. 3 we plot a graph of the fractal dimension D_f of the interface of the tumors with normal cells as a function of σ. D_f was calculated using a box counting algorithm [6]. The figure 3 shows an increase of D_f with σ and a tendency to saturation with D_f very close to the maximum value 2, implying that for large σ cells at the boundary are completely scattered. It is interesting to note that for σ = 0 the fractal dimension is slightly larger than 1 implying that the growth dynamics itself (without random adhesion), only cell division and rearrangement, contribute to tumor roughness.

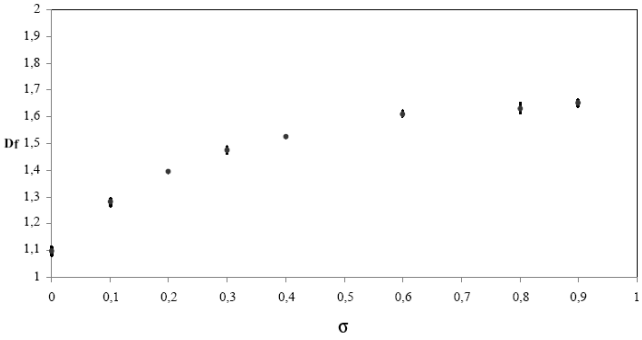


Fig.3.Plot of the fractal dimension of the boundary

Roughness is a useful quantitative measurement of the irregularity of an interface. Here we define it as the root-mean-square of the radius of a tumor. Tumor roughness *w* is defined as

$$w = \sqrt{\left(\frac{1}{L}\sum_{i}^{L}\left(r_i - < r >\right)^2\right)} \qquad (2)$$

where L is the length of the boundary, r_i is the distance (from the center of mass of the tumor) of a boundary cell and $< r >$ is the average radius of the tumor [10]. In Fig. 4 we plot the growth exponent β as a function of σ. As expected, β grows with σ. It tends to saturation close to β ~ 1.2.

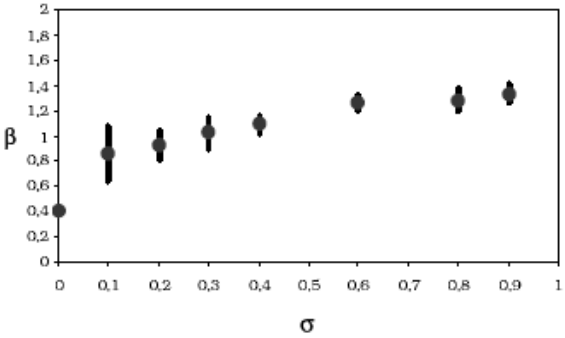
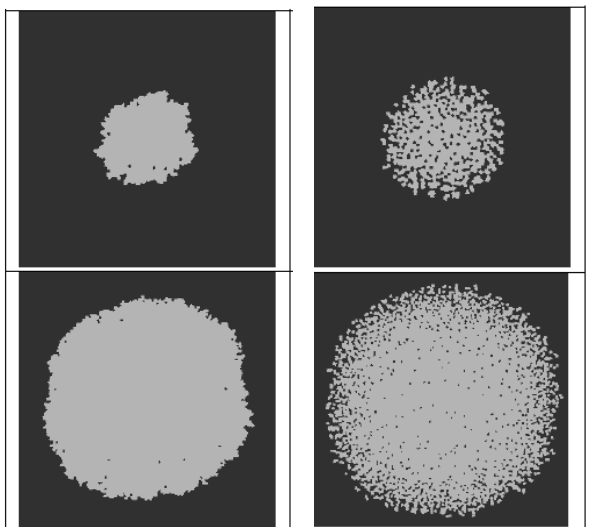


Fig. 4. Plot of exponent β as a function of σ.

As a conclusion, let accept that σ is a measure of the level of error on the forces of the tumor cells associated to the error in the expression of the adhesion molecules in real cells. σ = 0 implies that the adhesion force is expressed correctly and the only abnormality of the tumor is the high rate of cell division. In this case, we define the tumor as benign. For σ > 0 the roughness of the tumor increases and some cells start to detach from it. In this case we define the tumor as malignant. In figure 5 we show the simulated patterns for two different values of σ and for two stages of growth.



(a) (b)

Fig. 5. Simulated patterns for (a) σ = 0 (benign) and (b) σ = 0.9 (malignant) in two stages of growth.

The approximate number of tumor cells in the early and late stages is around 500 and 5000, respectively.

4 Experimental results

The simulations are conducted on a 200/200 square grid, with each cell initially defined to occupy a set number of lattice points, equal to the cell's target area, set in all simulations to be 20 pixels. The choice of parameter values in the simulations is determined by the relationship which we wish to study. To quantify the effect of the different parameters in the model on the invasion process, we concentrate on the parameter d_{max} which corresponds to the maximum depth of invasion (in pixels) on the grid. Biologically, d_{max} is an appropriate parameter to study as the maximum depth of invasion corresponds to the clinical severity of the disease, the likelihood of metastasis having occurred, and the options for clinical management. Our initial conditions throughout consist of a layer of cells at the top of the grid 10 cells thick with an initial (target) volume of 20 pixels. The boundary conditions are zero flux at the top and bottom of the grid and periodic at the left and right sides. This corresponds to a spatially extended lesion which is invading from an epithelial cell lining down through its basement membrane and into the surrounding stroma. Zero flux boundary conditions at the top are appropriate as cell masses of this type are usually localized in the epithelial boundary layers coating a lumen, so there is no tissue for the cells to move into in that direction. Periodic boundary conditions laterally are also appropriate in our simulation as the model is intended to examine a section through a spatially extended lesion much larger in size than could reasonably be modeled on our grid. Assuming that the cells are of a size of the order of 10 μm, the domain corresponds to a physical size of around 0.4 mm. Even the smallest detectable malignant lesion has a spatial extent considerably greater than this, which underlines the fact that the domain should be regarded as only a part of a much larger lesion. Periodic boundary conditions laterally help reduce boundary effects. Zero flux conditions at the base are arbitrarily set as such, as this is the limit of the validity of d_{max} in our model; hence, we stop the simulation before the bottom of the grid is reached.

The same model can be used to simulate proliferation. Malignant cells have a higher proliferation rate relative to their normal counterparts. Cells are triggered to divide by intracellular cascades which start when membrane-bound integrin receptors bind to extra-cellular matrix proteins. In our model, these adhesiveness (and the corresponding number of cell surface receptors) is quantified through the coupling constants. The alteration in d_{max} due to the inclusion of proliferation in the simulations is illustrated in fig. 6.

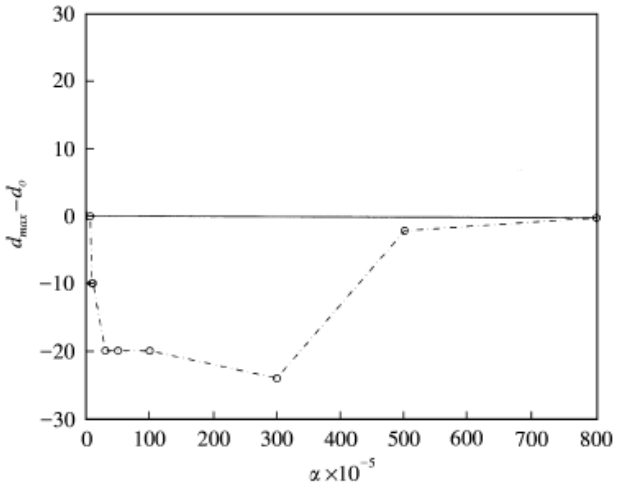


Fig. 6. Comparison between the depth of invasion including or not including proliferation

Intuitively, one expects proliferation to be proinvasive, on the basis that the additional cell population will facilitate invasion. However, as one can see, within some regions of the parameter space d_{max} is reduced due to proliferation. The explanation for this counter-intuitive result is related to the morphology of the advancing front. The front is created when fingers of invading cells join together to form an invading cell mass, when then break its contacts with the main cell mass behind it and moves on through the extra-cellular matrix. By including proliferation in the simulation, the fingers of cells

which initially ‘‘anchor’’ this advancing front to the main cell mass are thicker (as the cells comprising them are dividing) and also remain connected to the advancing front for longer (due to cells being pushed forward by their dividing and growing neighbors). The evidence for this is illustrated in fig. 7, which shows the effect of including proliferation on the morphology and depth of invasion of the invading cells (upper side) in comparison with the situation (down side) without proliferation (apart from the inclusion of proliferation, all other parameters are the same). The images to the left are obtained after 500 time units (actually computing iterations) while the images to the right are obtained after 2000 time units. Cells at the front are spreading out laterally to form an invading cell mass, and this mass is connected by long, thick fingers of cells to the main cell mass behind it. In the simulation with no proliferation included, these fingers are not present after the same length of time: they have already broken and the cells composing them have been pulled (under the influence of cell–cell adhesion) into either the cell mass in front of or behind them (depending on their position in front of or behind the point in the string at which the effects of haptotaxis and cell–cell adhesion balance).

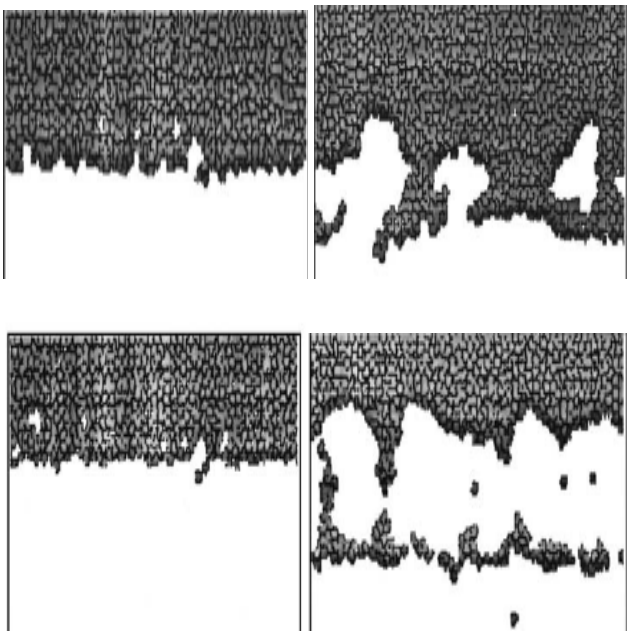


Fig. 7. The effect of including proliferation on the formation of the advancing front

Hence, one of the potential effects of including proliferation is to reduce the depth of invasion of a cell mass, although this possibility occurs only in a narrow region of the parameter space.

5 Conclusions and future work

The motivation for this work was to determine the relative importance and interrelationships between some of the main parameters involved in the invasion process, concentrating in particular on the influence of changes in cell–cell adhesiveness. In our model, changes in the adhesiveness between cells and the extra-cellular medium has a greater impact on the invasiveness of the cell mass (using the maximum depth of invasion after a given time as our index of invasiveness) compared with changes in cell–cell adhesiveness. The simulations show that a decrease in cell adhesion is sufficient to explain tumor roughening and cell detachment. Both result from cell displacement due to a locally random gradient of adhesion that increases outwards from the surface of the tumor and is controlled by the standard deviation σ. This supports the hypothesis that this process is important in the tumor transition from benign to malignant. In the same time it was shown that tumor boundary fractal dimension and roughness increase with σ and consequently fractal dimension can be an useful discriminator between the benign and malignant state of the tumor. Moreover, the results show that the roughness exponent of the tumors do not saturate and grows indefinitely with tumor size. In this case the useful parameter is the growth exponent β that seems to saturate close to 1.2. The inclusion of proliferation in the simulation showed that the morphology of the invading cell mass was changed by this inclusion, usually resulting in the cells invading as a solid mass rather than as a succession of ‘‘fingers’’ spreading out into the extra-cellular medium. However, for some regions of the parameter space, including proliferation resulted in a reduction in the invasiveness of the tumor.

In the near future we will focus our attention on the improvement of the simulation model, by adding other control parameters, such as the protease expression rate and the coefficient of haptotaxis. The assumption that in same cases an increased proliferation rate can lead in a reduction of the tumour’s invasive potential should be sustained by an experimental investigation using in vitro assays. In the same time it would be an interesting research study to determinate the therapeutic significance of our conclusions. Therapeutic interventions aiming at modulating the adhesive properties of the tumor have not attracted much attention, but in the light of our results we can make some predictions about the possible success of any such intervention which may

be developed. Such therapy should concentrate on strengthening cell–cell adhesion while inhibiting cell–ECM adhesion, and could be coupled with an additional intervention which inhibits the ability of the tumor to secrete proteolytic enzymes. In doing so, the cells will be more inclined to remain within the body of the main cell mass, as they will be held there through being tightly bound to their neighbors and through the absence of ECM gradients. The development of an intervention which blocks the cell–ECM receptors and thus reduces cell–ECM adhesion while failing to trigger the intracellular cascades which are believed to promote both proliferation and enzyme secretion may be an optimal strategy for inhibiting malignant invasion.

ACKNOWLEDGEMENTS
We acknowledge useful discussions with P. Flondor, V.Herlea, M. Rusu, M. Olteanu, D.Grecu, D. Crisan. We also thank Al. Lazeanu for providing us with the patterns of breast tumors. This work was partially supported by the Romanian Ministry of Education and Research under Grants No. 1467A/2005 and No. 2969/2005

References:

[1] J.C.M. Mombach, Universality of the threshold in the dynamics of biological cell sorting, *Physica A*., **276**, 2000, p. 391-400.
[2] R.M. Rangayyan, N.R. Mudigonda and J.E.L Desautels, Boundary modelling and shape analysis methods for classification of mammographic masses. *Med. & Biol. Engineering & Computing*. 13, 2000, p. 263-274.
[3] G. Landini, and J.W. Rippin, How important is tumour shape? Quantification of the epithelialconnective tissue interface in oral lesions using local connected fractal dimension analysis. *J. Pathol*., **179**, 1996, 210-217.
[4] R. Dobrescu, M. Dobrescu and F. Talos, Using fractal dimension in analysis of digital images, *Proc. of IASTED Int. Conf. VIIP*, 2002, p.57-63
[5] C. Vasilescu, V. Herlea, F. Talos, B. Ivanov and R. Dobrescu, Differences between intestinal and diffuse type of gastric carcinoma: a fractal analysis, In: Dobrescu R, Vasilescu C, eds. *Interdisciplinary Applications of Fractal and Chaos Theor*y, Editura Academiei Romane, Bucuresti, 2004, p. 144-149
[6] R. Dobrescu, M. Dobrescu and F. Talos, Multifractal medical image analysis using fractal dimension. In: Dobrescu R, Vasilescu C, eds. *Interdisciplinary Applications of Fractal and Chaos Theor*y, Editura Academiei Romane, Bucuresti; 2004, p. 78-83
[7] A. R. Anderson, M. A. Chaplain, E. L. Newman, R.J. Steele and A.M. Thompson, Mathematical modelling of tumour invasion and metastasis, *J. theor. Med.* 2, 2000, p. 129–154.
[8] S. Turner and J. Sherratt, Intercellular Adhesion and Cancer Invasion: A Discrete Simulation Using the Extended Potts Model, *J. Theor. Biology*, **216**, 2003, p. 85-100
[9] F. Graner and J. A. Glazier, Simulation of biological cell sorting using a two-dimensional extended Potts model. *Phys. Rev. Lett.* **69**, 1992, p. 2013–2016.
[10] A.-L Barabási and H.E. Stanley, *Fractal concepts in surface growth*, Cambridge, New York, 1995.