Non-linear model of cancer growth and metastasis: a limiting nutrient as a major determinant of tumor shape and diffusion

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Summary A new approach for modelling the spatio-temporal evolution of tumors is presented. To test its validity, a very basic model is considered, which, in spite of its simplicity, is capable of generating a multiplicity of morphologies and growth and migration rates. From an in-vivo scenario of basic life processes, cancer cell proliferation is described as a competition for basic nutrients. The chosen mathematical treatment and simulation techniques permit a direct implementation of the local nonlinear couplings existing between the various cell populations and the free and bound nutrient concentration. A discussion of the results and proposed improvements and applications of the model is also presented. © 1999 Harcourt Publishers Ltd

1. INTRODUCTION

The most striking feature of cancer is its ability to proliferate indefinitely without differentiation. That means that cancer cells behave like a unicellular organism whose growth is limited only by the environmental supply of nutrients and not by negative regulatory factors like hormones or cytokines, whose main role is to coordinate growth and differentiation of the various organs of the body.

Essential nutrients for eukaryotic cells include some aminoacids, glucose and transition metals like iron, zinc and copper. In the animal body the nutrients are not freely accessible to the cell, but they are supplied to the organs through the vascular system, which is also devoted to oxygen transport. Oxygen is a limiting factor for many cellular functions, including mitochondrial synthesis of ATP, heme synthesis, hormone hydroxylation and so on. It does not restrict, however, cell proliferation: enzymes involved in DNA duplication do not require oxygen and cancer cells typically have a very small number of mitochondria and produce lactate as a terminal product of glucose breakdown, instead of CO2 and H2O (2). As the efficiency of glycolytic pathways in synthesizing ATP from glucose is much lower than in mitochondrial respiration, the glucose requirement is much higher in cancer cells than in normal cells, making them strongly dependent on glucose supply for proliferation.

Nutrients introduced with the diet are carried through the vascular system by the blood, cross the vessel walls and then diffuse through the tissue to the individual cells. Since tumor growth is often very fast, the vessels are unable to grow at the same rate and a relative deficiency of blood supply develops. In this situation tumors are able to produce substances that increase blood flow or induce vessel proliferation, but usually this response is not sufficient to cope with the metabolic needs of the tumor and therefore the blood supply becomes a constraint (3).

Additional limiting factors are: (a) its anatomical location between unextensible tissues (bones, muscles, tendons, skin); (b) the competition for nutrients with other cell populations, such as lymphocytes or macrophages (cells of the immune system) infiltrating the tumor (4).
According to the most recent theories, what we call life cannot be described by linear equations but only as a temporal sequence of discrete events, with the choice at every bifurcation depending on the local conditions at the time. A suitable model for life-related events like cancer growth must therefore

- include a dependence on the environmental variables and cell population properties;
- introduce stochastic fluctuations of the variables within preordained ranges;
- simulate through a large number of cycles the temporal sequence of life events.

To develop a model as general as possible, one needs a deep knowledge of the rules driving cell life, modelling at first only some very simple processes. As a second step one increases the complexity of the system by introducing evolution into each parameter, and checking the behavior of the model after each evolutionary step. This approach is expected to mimic the evolution of the living world, attempting a correspondence with real life.

The obvious interest of the problem under consideration has led to the formulation of increasingly sophisticated mathematical models for tumor growth. An excellent survey of the state of the art in tumor modelling may be found in a recent book edited by J. A. Adams and N. Bellomo (5). The philosophy of the model presented here is somewhat different from that of previous mathematical models. By paying close attention to the local evolution and coupling of a few relevant variables, we sacrifice the possibility of obtaining nontrivial analytical solutions. In exchange, we can model closely some of the crucial events taking place at the cellular level and simulate numerically the spatio-temporal evolution of the system.

In the next section, we discuss an in-vivo scenario, which can be viewed as a biochemical basis for the mathematical model described in Section 3. The model leads us to a simulation of the spatio-temporal evolution of the system, which will allow us to follow it from its start, idealized as a lump of seminal cells, up to an unre lenting growth or to a metastasis or, eventually, to a latent state. The mathematical tool for the numerical simulation will be provided by the local interaction simulation approach (LISA). LISA has already been successfully applied to study the spatio-temporal evolution of a variety of phenomena, such as wave propagation (6,7), diffusion (8), absorption and desorption (9) and growth (10).

LISA is well suited for parallel processing, which becomes very important for a detailed solution of large-scale problems. In the present contribution, we limit ourselves to demonstrate the suitability of our approach and its capability to reproduce on a small grid the essential features of cancer growth by means of a few simple examples, which are discussed in Section 4. A more detailed study, involving simulations of specific neoplasms and comparison with clinical data, is in progress.

2. AN IN-VIVO SCENARIO

When the amount of nutrients is limited in a specific environment (bacteria in the soil, trees in a forest), the growth rate of every population is strictly dependent on its ability to compete for essential nutrients in the ecological niche. To abate competition many organisms have developed different electron acceptors (oxygen or nitrate) for respiration and generated strategies to use different sources of energy, such as light (plants, photobacteria) or carbon skeleton molecules, e.g. different aminoacids, sugars or fatty acids (animals, fungi, bacteria). However, all living organisms retain a common need for certain very basic elements, notably such transition metals as iron, zinc and copper.

Iron is the most representative among these transition elements: it is involved in oxygen transport, in many redox reactions, including mitochondrial electron transport, in all the hydroxylase reactions, in the uric acid synthesis and in the synthesis of deoxyribonucleotides from ribonucleotides (ribonucleotide reductase (RR). Since the latter is essential for DNA synthesis and, therefore, for reproduction, iron is a requisite nutrient for all living organisms (11).

While Earth is more than 30% iron, iron concentration is much lower on the earth surface, where it is found mostly as iron oxide, due to its strong reactivity toward oxygen. The pool of biologically available iron is therefore limited and thus becomes the most critical growth-restricting factor. As free iron is highly toxic for living organisms, iron uptake by the cells is mediated by special carriers called siderophores, whose synthesis is regulated by the actual cellular need of iron. Cells may produce one or more siderophores, with different affinities for iron; the highest the affinity, the strongest the evolutionary advantage for the species. Siderophores differ in chemical structure from simple molecules like citrate, to amnicoid derivatives (hydroxamates, polyphenols), to proteins (transferrin, melanoferrin) (12).

On the basis of the pattern and amount of siderophores produced by the cells and the number of corresponding receptors, it is possible to forecast the growth rate of the cell population under study assuming that the iron availability in the selected environment and the ability of the competing populations to produce siderophores and/or receptors are known.
Cancer cell proliferation can be described as a special case of the general scenario outlined before, by applying the following subset of rules:

1. all the cells belong to the same species and use the same siderophore ( transferrin for humans);
2. cancer cells display a higher number of receptors for Tf ( TFR) and therefore a higher affinity for transferrin than the other cells of the same organism;
3. the environment in which cancer cells live has a limited supply of iron from blood circulation. Iron supply is therefore strictly dependent on the number, size and functional state (vasodilation or vasoconstriction) of the local vessels;
4. some groups of cells can develop a growth rate and iron affinity comparable to those of the cancer cells (immune system);
5. both cancer and immune cells may produce small peptides ( cytokines), which can increase the growth rate of the producer and depress the growth rate of the competitor;
6. cancer cells can reproduce, die or diffuse. It is well known that cancer cells reproduce when enough iron is available (13). They are also very sensitive to lack of iron or to treatment with iron chelators like deferoxamine (14). Healthy cells stick together through adhesion molecules, whose expression is regulated by cytokines and iron (15). Cells treated with iron chelators first detach and (twelve or more hours) later undergo programmed death (apoptosis). Their intermediate reaction (before death) to a sharp iron deficiency is detachment and diffusion.
7. when a cancer cell enters the blood flow, it can diffuse everywhere. The organs where cells stop most frequently ( metastasis) are bone marrow, liver and lung: these tissues have the highest iron concentration in the whole organism and the cells to which metastatic cells stick are preferentially macrophages well fed on iron (16).

3. THE MODEL

In order to reproduce the basic features of cancer growth, as discussed in the previous section, we consider the following model. We assume that the region of interest may be confined to a slab of tissue, which for simplicity we consider two-dimensional and rectangular. We also restrict ourselves to considering a single nutrient, i.e. iron, as the pivotal ingredient for cancer cells growth. To mimic the anatomic vascular constraints for its supply, we assume that a single blood vessel runs along one of the sides of the slab, delivering free iron to the tissue.

We then discretize the slab by means of an \( M \times N \) grid and call \( p_{ij} \) the density of free iron at the node \((i, j)\). We also discretize the time and call each time step \( \Delta t \). We then write the master equation for the free iron diffusion and absorption ( consumption) by the tissue

\[
\frac{\Delta p_{ij}}{\Delta t} = \alpha (\sum_{n.n.} p_{n.n.} - 4p_{ij}) - \beta p_{ij}
\]

where \( n.n. \) represent the nearest neighbours of \((i, j)\), i.e. \((i \pm 1, j)\) and \((i, j \pm 1)\), and \( \alpha \) and \( \beta \) are the free iron diffusion and absorption rates, respectively. Eq. 1 must, of course, be complemented by proper boundary conditions.

Next we assume that each grid node \((i, j)\) may include a number \( h_{ij} \) of ‘ healthy’ cells, a number \( c_{ij} \) of cancer cells and a number \( d_{ij} \) of dead cells. For simplicity we normalize \( n = h_{ij} + c_{ij} + d_{ij} \) to one. We also call \( q_{ij} \) the global amount of ‘ bound’ iron belonging to the \( c_{ij} \) cells in \((i, j)\). We assume, as initial conditions, that \( d_{ij} = c_{ij} = q_{ij} = 0 \) everywhere, except at a given node \((i, j)\), which corresponds to the location of the tumor seed at the time \( t=0 \). There we take \( c_{ij} = c_{0} \), \( q_{ij} = q_{0} \).

We then set the rules of the game by means of the following five basic processes:

1. feeding: free iron is transformed into bound iron by cancer cell uptake at a rate \( \gamma_{ij} \). This transformation generates a nonlinear coupling of the equations for \( c_{ij} \) \( p_{ij} \) and \( q_{ij} \). For low concentrations of free iron, \( \gamma_{ij} \) is directly proportional to \( p_{ij} \), \( \gamma_{ij} = \gamma p_{ij} \). For higher concentrations, \( \gamma_{ij} \) saturates and becomes nonlinearly dependent on \( p_{ij} \). This situation is well described if we choose \( \gamma_{ij} = \gamma(1 - \exp(-p_{ij})) \). By contrast, the healthy cells continue to absorb iron at a fixed rate \( \beta \);
2. consumption: bound iron is utilized by cancer cells at a rate \( \lambda_{ij} = \lambda(1 - \exp(-q_{ij})) \);
3. death: if, at any time and grid location \((i, j)\), the average amount of bound iron per cell \( q_{ij}/c_{ij} \) falls below a given threshold \( Q_{D,i} \), a random number of cancer cells \( r_{ij} < c_{ij} \) die in the subsequent time step;
4. mitosis: if, viceversa, \( q_{ij}/c_{ij} \) becomes larger than a given threshold \( Q_{M} \), a random number \( r'_{ij} < c_{ij} \) of healthy cells become cancerous;
5. diffusion: if too little free iron is available in \((i, j)\), i.e. if \( p_{ij}/c_{ij} \) falls below a given threshold \( P_{o} \) then a random number \( r''_{ij} < c_{ij} \) of cancer cells migrate to neighbouring nodes, looking for ‘ richer pastures’. We call their diffusion rate \( \alpha \). Note that, when cancer cells move, they take their bound iron along.

These rules lead to a set of iteration equations, which we omit here for brevity. We also omit the discussion of a number of details, which must, however, be specified in the actual implementation of the model, such as the sequence of application of the above processes and the choice of \( r_{ij} \), \( r'_{ij} \) and \( r''_{ij} \).
4. RESULTS AND DISCUSSION

The model proposed in the previous section allows us to obtain two-dimensional images of the growing cancer. In the present simplified version it does not incorporate border effects, due, e.g. to anatomical constraints. Therefore it may be applied only to tumors growing in the brain and other soft tissues. In fact, as we will see in Figure 1, the morphological correspondence with real brain tumors (2) is quite good. This type of cancer usually displays a spheroidal shape with a necrotic center and seldom gives rise to metastasis. With our model we can get different kinds of spheroids by slightly modifying the ratio supply/affinity of iron. Altering further this ratio or varying other basic biochemical parameters, such as response to nutrients availability and free iron diffusivity, it is possible to arrive to radically different outcomes, as demonstrated by our numerical analysis.

The best way to visualize the spatio-temporal evolution of a tumor is through a movie, which gives a continuous representation of its form and size. It is also possible, of course, to represent the evolution of the propagation of the population of the healthy and/or necrotic cells. The results shown in our figures are snapshots of the concentration of cancer cells taken from such movies. Higher concentrations are depicted in lighter gray tones. For each set of snapshots we include a graph showing the time evolution of the average concentrations of cancerous and dead cells and the total number of cancer cells that have migrated into the blood vessel. The latter may be responsible for metastasis provided, of course, that the damage to the tissue at the time has not yet proven fatal.

In all figures the tumor seed is created at time t=0 at the specimen center with an initial value $c_0 = 0.2$. The nutrient source (‘vessel’) is located at the left edge of the specimen; there the nutrient concentration is kept to a fixed value $p_0$. Periodic boundary conditions are chosen for the upper and lower edges. The nutrient concentration at the right edge is initially assumed to have a given value $p_r$ (it will later change as the tumor evolves). The initial free iron concentration, which is given by the steady state solution to Eq. 1, is a gently decreasing function of the distance from the blood vessel. The parameters that are kept constant in all the figures are given in Table 1.

Table 1 Parameters corresponding to the simulations reported in the paper

<table>
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<tr>
<th>$c_0$</th>
<th>q0</th>
<th>pr</th>
<th>$\gamma$</th>
<th>QM</th>
<th>QD</th>
<th>PD</th>
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In Figure 1, the nutrient availability (i.e., the concentration at the source is $p_0 = 0.5$ and the nutrient consumption rate is $\lambda = 0.03$. Since the nutrient gradient is very low, the tumor grows almost isotropically. The inner dark region in the three last snapshots is almost completely necrotic. True cancer activity is confined to the peripheric (lighter) region. The low overall nutrient availability is responsible for the very fast rates of growth and death, since cancer cells aggressively search for nutrients and rapidly exhaust the scarce available supplies. Note that a sizable quantity of dead cells is already present at time $t = 240$ and that the cancer has reached the blood vessel by $t = 500$. The high volume achieved by the tumor when it reaches the vessel will probably make the patient survival and, consequently, the possibility of metastasis, unlikely. The concentration plots show that the number of cancer cells in the tumor
attains a maximum when the tumor reaches the nutrient source. At that moment it starts decreasing, while the number of cancerous cells migrating into the bloodstream grows rapidly. The number of dead cells stabilizes shortly thereafter (about 40% of the tissue is by then dead).

When the nutrient consumption rate becomes higher ($\lambda = 0.1$) with the same amount of nutrient availability ($p_0 = 0.5$), a ‘dormant’ tumor may form, as depicted in Figure 2. The tumor grows slowly and isotropically in the first stage of the simulation. By $t = 200$, it has reached a quasi-stable condition, in which nutrient availability and consumption rates compensate. The tumor shape is only slightly modified, as shown by the snapshot at time $t = 2000$. A small increase in the consumption rate would have led to the complete death of the tumor.

Keeping $\lambda = .1$ but doubling the nutrient availability ($p_0 = 1$), a very different morphology arises (see Figure 3). In the early stages, tumor growth is slow because the cancer cells record high local nutrient concentrations and do not need to move to find additional iron elsewhere; therefore, their diffusion is slow. On the other hand, the high iron consumption limits the mitosis rate. Therefore, the growth is not so malignant per se as that shown in Figure 1 (note the darker hues of grey depicting the active cancer cell domain in Figure 3). At $t = 300$, the cancerous tissue, sensing the relatively high nutrient distribution gradient, starts to grow anisotropically. Again a necrotic core develops in the region confined by the active tumor cells. At $t \approx 1000$, when the tumor reaches the source neighborhood, its growth becomes explosive. This can be seen by noting the brilliant frontal region of the growing tumor in the $t = 1450$ snapshot and in the concentration plots. From these plots we also see that, by the time the tumor reaches the vessel, only a small proportion of the tissue is affected; consequently, the probability that metastasis will occur is very high.

Figure 4 represents the results obtained for a simulation with $p_0 = 3$ and $\lambda = 0.1$. The high availability of nutrient and its strong distribution gradient lead to a very fast, malignant and anisotropic growth around a completely necrotized core. Despite the high diffusion speed of the tumor (reaching the vessel slightly after $t = 600$), the occurrence of metastasis is unlikely, due to the fast and strong growth of both cancerous and necrotic tissues (less than 50% of the specimen is healthy when cancer cells start migrating into the vessel.)
5. CONCLUSIONS

Although in their early stages all tumors exhibit compact spheroidal shapes, there are multiple paths for their later evolution, which are determined by the tumor specific nature and location. In this paper we have proposed an approach that is suitable to describe all growth patterns and shapes found in clinical experience. Using the basic elements contributing to tumor development at the cell level, we have presented a description that is general enough as to permit its ready implementation in a manifold of particular instances. To exemplify the proposed approach, we have formulated a simple mathematical model, in which we have considered a single nutrient, no inhibitors and no inhomogeneities in the diffusivities. The predictive power of even this simplified formulation is apparent from the figures. By solely varying two biochemically relevant parameters between reasonable bounds, we have shown that the model leads to a wealth of possibilities for neoplastic development. The figures also demonstrate the crucial roles played by the nutrient consumption rate of the cancer cells and by the nonuniformity in the free nutrient distribution. This nonuniformity, which is initially only a function of the distance from the nutrient source, is strongly modified by the presence of cancerous tissue and is, in turn, pivotal in determining tumor morphology and activity.

An advantage of simulating the process through a locally sensitive approach such as LISA is that any modification or improvement of the model becomes much easier to implement than through the usual differential equation formalism. E.g. tissue inhomogeneities give rise to position-dependent diffusivities and vascularization may lead to a complex source geometry. These features may be trivially included in a LISA approach. It is also easy to incorporate the action of any other growth modulators, such as cytokines, the competition with other cell populations or the recycling of consumed nutrients.

To conclude, we remark that, from the general discussion of Section 2, cancer evolution appears to proceed stochastically, while the model realization of Section 3 is essentially deterministic (except for the presence of $r_1$, $r_2$ and $r_3$). In fact, this model may be thought of as having some weak stochasticity, which is, however, washed out in the course of many iterations, so that it does not appear explicitly in the equations. Stronger stochasticity may be directly included to enrich the model; this interesting possibility will be explored in a subsequent paper. We also intend to apply the approach presented here to specific clinical cases. By including other features, as necessary, to make the model sufficiently realistic, we are confident that our simulation technique will help in identifying the key molecular processes that determine the course of different neoplasias. In addition, by explicitly introducing a local mechanism for the action of various chemical or radiological agents, our approach might also be used to perform ‘computer experiments’, which could lead to the optimization of the therapy.

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