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Stochastic modeling of tumor induced angiogenesis in a heterogeneous medium, the extracellular matrix

Franck Amyot, Alex Small, Amir H Gandjbakhche

Lab. of Integrative and Medical Biophysics, National Institute of Child Health and Human Development (NICHD)
National Institutes of Health (NIH), Bethesda, MD 20892, USA

Email: amyotf@mail.nih.gov

Abstract: **Angiogenesis, the formation of blood vessels, is a process whereby capillary sprout are formed in response to external stimuli. We model the tumor induced angiogenesis on keys events such of migratory response of endothelial cells to tumor angiogenic factors and the local cell interaction with the extracellular matrix (ECM). We consider the ECM medium as a statistically inhomogeneous two-phase random medium.**
Numerical simulations of the model are presented. Using this model, we will compare the influence of ECM distribution on vascular network formation. By developing mathematical models of angiogenesis, we hope to provide a deeper insight into the mechanisms underlying angiogenesis.

Keyword: angiogenesis, cell migration, apoptosis, percolation, vascular network.

I. INTRODUCTION

The migration of vascular endothelial cells (ECs) plays an important role in tumor-induced angiogenesis [1, 2]. ECs are precursors to new capillaries formation [3, 4] and form the lining of all blood vessels. The migration of ECs allows movement and growth at the sprouting tip of new capillaries [5, 6]. In this process, cell migration is regulated by two major forces: chemotactic forces [7] that lead the cell to the tumor depending on the concentration gradient of several growth factors, and haptotactic forces [8, 9] due to local adhesion between the cell and the extracellular matrix (ECM) via integrins [10]. Local ECM mechanical signaling is essential for capillary formation. Depending of the type and distribution of matrix protein inside the ECM, the ECM controls also the EC apoptotic response and EC differentiation [11-13].
Previous models considered the ECM as a homogeneous medium. In reality, the ECM is a highly heterogeneous network of composed of polysaccharide chains, fibrous proteins (collagen and elastin), fibronectin, and laminin. In fact, the ECM can contain traps for integrins and possess different fiber orientations that affect capillary morphogenesis [14]. This heterogeneity influence the local physical and chemical proprieties of the ECM (i.e. diffusion coefficient and chemotactic and haptotactic factors). The ECM mesh also gives support to cells, and allows them to proliferate and self organize into a vascular network [15, 16]. In the same way, local properties of the ECM induce cell signals for many integrins, fibroblasts, and extracellular proteolytic enzymes. Moreover, local disruption of the ECM induces cell apoptosis with adjacent cells and regulates the vascular morphogenesis [14].
We present a stochastic model for tumor vascularization which takes into account the heterogeneous nature of the ECM. We model the ECM as a two-phase random medium where each phase represents the local EC response to ECM. If locally the response is to EC apoptosis, then this area ECM will not be able to support the capillary formation. Regardless of the distribution and the number of these areas, we will study the perfusion and the vascularization of tumor.
Our stochastic simulation is composed of a random walk model where the probability of a sprout moving in a given direction is associated with the EC's chemotactic and apoptosis response in this particular direction. Our model also simulates also branching and anastomoses so that we create real vascular network. Our results show different degrees of tumor perfusion or vascularization, depending on the ECM heterogeneity. We present a new look at the geometry of the ECM and how this geometry regulate angiogenesis

II. MATHEMATICAL MODEL

We define three variables in the model. Capillary presence is represented by an indication function, n (with a value 0 or 1 depending of the presence or not of the capillary). Profile of TAF concentration between the tumor and the capillary is represented by c(x,y). The heterogeneity of the ECM is represented by a two phase medium and an indication function, $d_{i,j}$ (with a value 0 or 1 depending of the possibility for the ECM to sustain sprout formation).

a)Geometry

A classic tumor Folkman model was used: a capillary segment of length d and a tumor area located at a distance d from the capillary. The domain is a square of surface d^2, the tumor is at the top center of the domain (half-circle of radius to d/20) and the capillary at the bottom (Figure 1a). All of the boundaries have no flux conditions for cells and TAF (symmetric conditions).

b) Tumor angiogenic factor transport

Angiogenesis begins with the release of a tumor angiogenic factors (TAF) by tumor cells. In our simulations, we consider the TAF concentration profile around the tumor is in a semi-steady state solution (figure 1b).

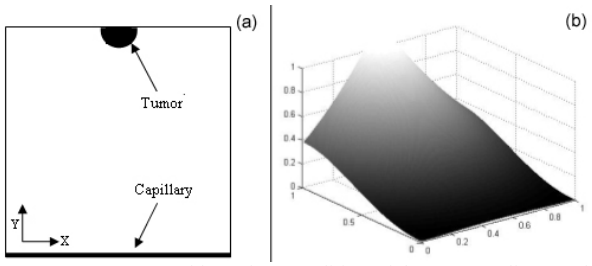

Fig 1: (a) Geometry and boundary condition of the TAF medium study. **(b)** TAF concentration profile approximates a gradient produced by a circular tumor source.

c) Sprout formation

Even if the direction of the sprout depends on the chemotactic response of the EC in a certain direction, it is the apoptotic response which allows the sprout to grow. The apoptotic response of the EC depends highly on the physical-chemical properties of the local ECM (rigidity, composition, and orientation of the ECM fibers). In our model, the ECM is a statistically heterogeneous, two-phase random medium. Some parts of this medium are able to support ECs and allow sprout growth while other parts cannot. The ECM is simulated by a square lattice where each site is a box of area ℓ^2 and is characterized by a uniform random probability p. If p is higher than p0, then the ECM is able to locally support the growth of a sprout. If p is lower than p_0, then this area does not allow capillary formation and the sprout cannot go in that direction. The ECM surface fraction (Φ) is defined by the surface in the domain that can support capillary growth divide by the surface of the domain.

The growth of the sprout is based on a walk model based on differential equation. The motion of the sprout depends on five coefficients (P_i). Each coefficient gives the probability for the sprout to go in one of the four possible directions or to stay at the same position. These coefficients correspond to the random motility and to the chemotaxis response of EC in one direction. The resulting equation governing the migration of an endothelial cell has the form:

$$n_{i,j}^{t+1} = n_{i,j}^{t}.P_0 + n_{i+1,j}^{t}.P_1 d_{i+1,j} + n_{i-1,j}^{t}.P_2 d_{i-1,j} + n_{i,j+1}^{t}.P_3 d_{i,j+1} + n_{i,j-1}^{t}.P_4 d_{i,j-1}$$

During the formation of the vascular network, the sprout must be able to form anastomoses with other sprouts and also to create branches. It is the succession of these events that will create a real microvascular network:

-Two sprouts are assumed to form anastomoses when the tip of one sprout meets another sprout. In this case, the growth of the tip stops and the tip merges with the other sprout.

-A new branch appears from existing new tips and the division is due to the presence of TAF. In our stochastic model, the probability to have a branching is proportional to the concentration of TAF *($P_{branching}=\beta.C(x,y)$, $\beta=0.2$).*

III. RESULTS

From this stochastic model, we performed several simulations in order to study the development of capillaries around a tumor. We stress on the distribution of the ECM (for different surface fraction and tomography) on the possibility or not for the tumor to induced angiogenesis. The figure 2 represents a tumor vascularized form 5 initial sprouts. . At the end of the simulation, the tumor is vascularized by several sprouts, more than the five initials budding sprouts and a real vascular network surrounds the tumor We define the percentage that vascularize the tumor by the number of simulation which give a vascularized tumor on the total number of simulation. Here 2000 simulations have been made for each ECM surface fraction.

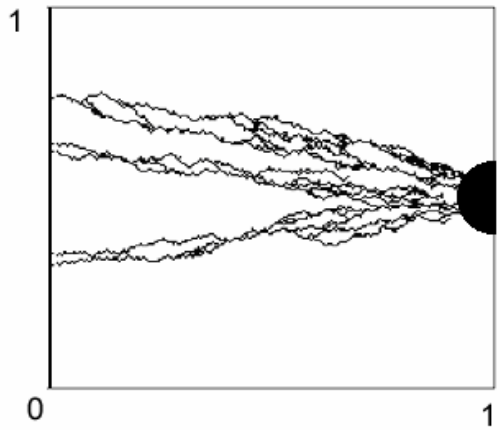

Fig. 2: simulation of a vascular network in a homogeneous ECM. The branching probability coefficient worth 0.2

Figure 3 indicates that the interaction between vascular network and the ECM is essential for angiogenesis. Angiogenesis requires a sufficiently dense ECM for the sprouts to reach the tumor. Under a fraction surface threshold, the tumor cannot create a vascular network. The value of the threshold is independent to the box size that defined the medium heterogeneity of the ECM.

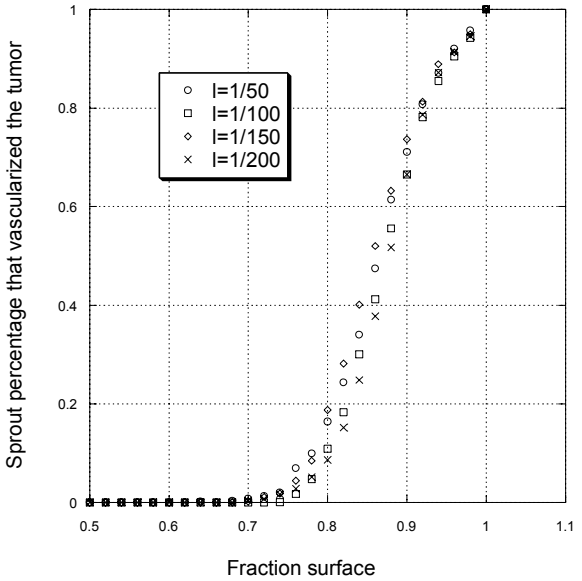

Fig 3.: Percentage of tumor vascularization for different ECM fraction surface density value. The simulations start with one initial sprout and the probability of branching equal 0.2. The simulations were made for different box edge (l).

This threshold value is 0.77±0.1. Below the threshold, the tumor vascularization is difficult. So, this heterogeneous distribution of the ECM creates a barrier for the angiogenesis.

Nevertheless, the distribution of domain that can support capillary growth is crucial for the vascular development. The figures 4a, 4b, 4c, 4d show four simulations for the same surface fraction (Φ=0.8) but different ECM topology. It's clear that during the growth of the vascular network, a competition exists between the attraction exerted by tumor and the preferred path created by the ECM.

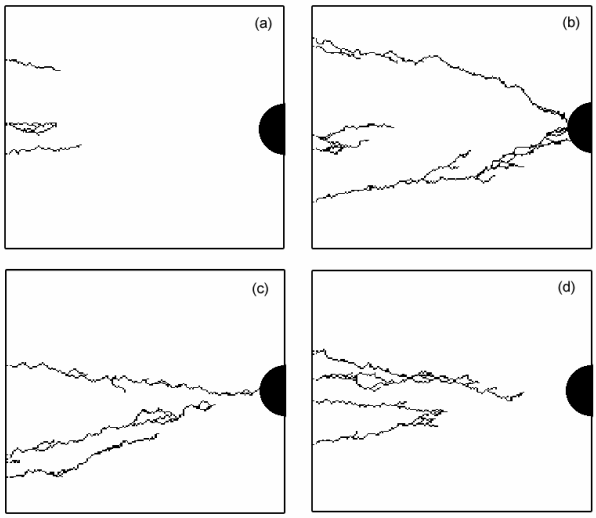

Fig 4.: Simulated vascular network for the same ECM distribution. The ECM fraction surface is 0.8 and the number of initial bud sprouts is 5.

IV. DISCUSSION

Analysis of vascular networks sprouting from a vessel segment is useful to understand the mechanisms underlying vascular morphology. The progression from a single cell to a capillary has showed how essential are cell/cell interactions and cell/ECM interaction are. These interactions caused by a sequence of signal that ultimately result in the formation of a mature capillary. But the morphology of a full vascular network surrounding a tumor is unclear. Different parameters have been used to characterize such networks (fractal dimension, vascular density) and analogies have been made with several model disordered systems. For Baish et al [17], a vascular network based on a percolation model explains why these networks have higher resistance than homogeneous one. In a percolation network, the fractal dimension is higher than normal vascular networks and is closer to tumor vessel network observed in vivo. Diffusion limited aggregation has been also a successful model for describing in vivo vascular network [18] even if the fractal dimension characterizing such networks seem to be closer to normal vascular networks rather than tumor vascular networks. Also an in vitro study have show that the fractal dimensions of cell network fit a diffusion limited cluster aggregation model and depends on the cell concentration [19], i.e cell proliferation will increase the network fractal dimension.

All of these models display properties similar to vascular network; nevertheless they are all are based on the assumption that the ECM is a homogeneous medium. The ECM is formed by several fibers like collagen and elastin. In the presence of integrin, ECs adhere to these fibers and the ECM mesh which gives the morphology of the network. In the same way, an in vivo study has shown that a preexisting basement membrane in the ECM is a favored path for the perfusion of the tumor by a new capillary [20].

The purpose of tumor-induced angiogenesis mechanism is for the vessels to perfuse the tumor. In this paper, the perfusion threshold value was given for different densities of ECM material. In other words, we determine the probability for a sprout to reach the tumor while migrating through a disordered system, i.e. the repartition of macromolecules in the ECM. This is similar to a typical percolation problem which consists of determining a threshold concentration that allows percolation from one edge to the other. Many studies have calculated, simulated or measured these values and these values depend on the nature of lattice. For example, with triangle randomly disposed this value is 0.6527 [21], 0.67637 for a "Swiss cheese" model [22] and 0.5927 for a square lattice [21].

Our value is to 0.77 for the vascular network threshold. This value is higher to the classical values found in literature because the tumor, by the TAF gradient surrounding it, excludes some pathways. From this, the ECM composition determines whether or not tumor-induced angiogenesis can occur.

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