

The study of angiogenesis effect on the growth rate of an invasive tumor using a mathematical model

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Abstract — A mathematical model describing the growth of an invasive tumor in a tissue is developed taking into account angiogenesis. Malignant cells in the state of metabolic stress generate a growth factor of vascular endothelium, which stimulates tumorous angiogenesis. The model takes into account the dichotomy of migration and proliferation of tumorous cells depending on the concentration of the nutrient. The numerical investigation of the model has shown that angiogenesis for invasive tumors does not practically affect the growth rate in a tissue. The cause and significance of this result are discussed.

According to the data of the World Health Organization, mortality related to malignant neoplasms takes the second place after mortality caused by cardiovascular diseases. Moreover, in contrast to the latter ones generally affecting elderly people, malignant tumors occur in people of all ages and are the most dangerous (lethal) for young patients. All this attracts much attention to the development and study of new methods of antitumor therapy, including methods of mathematical modelling.

In the course of its growth, a tumor undergoes a series of structure changes determining the rate and character of its development [8]. At the initial stage, a tumor is simply a colony of progressively dividing malignant cells, which determines an exponential rate of its growth. When the number of cells reaches several millions, the amount of nutritive material at the center of the tumor becomes insufficient for division. The cells pass to the rest state and then, as the tumor grows, a necrotic zone consisting of the

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remnants of dead cells is formed in its center. At this stage the radius of the tumor growth linearly in time [2].

As early as 1966, Folkman and co-authors showed that the pre-existing circulatory system provides the growth of an intertwined tumor in an isolated organ up to the radius of 3–4 mm [4]. A further growth requires neovascularization, i.e., formation of new blood vessels from the pre-existing vascular network. This process is also called the tumor angiogenesis. In 1971, Folkman proposed a new method of antitumor therapy, the so-called antiangiogenic therapy [5]. The standard chemical and radio therapies are focused on the destruction of actively proliferating cells, both cancer ones, and special organic cells, which leads to a whole set of negative side effects. The antiangiogenic therapy is aimed at blocking the tumor neovascularization, which should lead to an essential decrease in its growth rate, and ideally to its complete stop. It is assumed in this case that there is no negative influence on normal tissues. A considerable progress has been achieved in the last decade in the study of the mechanism of angiogenesis and the factors of its regulation. The basic (universal) substance stimulating angiogenesis is the vascular endothelial growth factor (VEGF). It has been shown that this protein molecule having the mass about 40 kDa is actively produced by different types of cells, including tumor ones in hypoxic conditions [20]. The intergrowth of new capillaries in a tissue occurs in the direction of zones with a high concentration of VEGF. It is assumed that this process consists of the following successive steps: enzymatic degradation of capillary basement membrane, endothelial cell (EC) proliferation, directed migration of ECs, tubulogenesis (EC tube formation), vessel fusion, vessel pruning, and pericyte stabilization [1]. In this case, neovascularization results in a condensation of the capillary network in hypoxic tissues, which should improve their supply with oxygen and other metabolites (glucose, lactate, etc.).

Tumor angiogenesis is also caused by the production of VEGF by hypoxic cells inside the tumor. However, experimental investigations have shown that the vascular network appearing in this case essentially differs from the normal one. The newly formed capillaries have a greater diameter, lower blood filling, and, moreover, there are large cracks in their walls causing blood leakage into the tumor and the tissues surrounding it. All these facts indicate that the capillary network formed as the result of tumor angiogenesis is inefficient [12]. Moreover, tumor cells produce various ferments in the process of their growth (in particular, EphB4), which breaks

the regulation of angiogenesis and thus most capillaries positioned inside the tumor are degraded and the walls of the remaining ones become thicker, hence preventing the percolation of nutritive materials into the tissue [17]. All this provokes questions concerning the role of angiogenesis in the tumor development. Is the effect of the newly formed capillary network on the supply of the tumor by metabolites essential, and which are the cases when we can neglect this? This problem has motivated studies using mathematical simulation of tumor growth subject to angiogenesis. It should be noted that initial works in this direction [15] modelled only angiogenesis itself, i.e., the formation of new capillaries, and the tumor was not considered as a stationary source of the VEGF. However, a series of papers appeared recently where the growth of tumor was simulated taking into account angiogenesis [16, 18]. We should admit that those models contain a lot of parameters which cannot be determined experimentally. This restricts the applicability of such models in simulation modelling. Varying the parameters, one can simulate the growth of a tumor subject to angiogenesis and qualitatively compare the results of simulation with data of magnetic resonance imaging and histological analysis of clinical patients. In spite of the fact that the results of such comparison demonstrate the qualitative similarity of the cellular and spatial structure of the real tumors and the simulation results *in silico*, the practical value of this approach is doubtful. In this paper we present a new mathematical model and use it for the study of the dependence of the invasive tumor growth rate on the rate of neovascularization of the tissue. From the practical viewpoint, such simulation can help in estimation of the possible antitumor efficiency of antiangiogenic therapy for metastatically active invasive tumors.

1. The model

The interrelation of the variables in the tumor growth model taking into account angiogenesis is presented in block diagram (see Fig. 1).

In this model the tumor is considered as a colony of cells surrounded by a normal tissue with a pre-existing vascular network. Living cells can exist in two following states: proliferating state with the density $n_1(r, t)$ and migration with the density $n_2(r, t)$, where r is the spatial coordinate, t is the time. The intensity of the transitions $P_1(S), P_2(S)$ from one state to the other depends on the concentration of the nutritive material $S(r, t)$: for a

high concentration the cells divide at the constant rate B and do not migrate. If the metabolite concentration essentially decreases, the cells stop dividing and begin to migrate randomly with the coefficient D_n searching for domains with a high level of nutritive substratum. The nutritive material comes from the vascular network, diffuses into tissues, and is consumed both by malignant cells (proliferating cells consume much larger quantities than migrating ones), and by normal organic cells with the density $h(r, t)$. We consider a thick incompressible tissue, so that $h(r, t) + n_t(r, t) = \text{const}$, where $n_t(r, t)$ is the total density of the tumor cells including dead cells $m(r, t)$ ($n_t(r, t) = n_1(r, t) + n_2(r, t) + m(r, t)$). We assume that the volumes of all cells are equal.

We have already used this approach in our previous paper in the simulation of the invasive tumor growth [11]. It is based on the principle of the dichotomy of migration and proliferation of tumor cells revealed experimentally [7]. If migration cells do not get to a domain with a high nutrient concentration, they die at the rate d_n . Based on the assumptions presented above, we can write down the following system of equations for the densities of the living and dead tumor cells and also for the nutritive material concentration:

$$\begin{aligned}
 \frac{\partial n_1}{\partial t} &= Bn_1 - P_1(S)n_1 + P_2(S)n_2 \\
 \frac{\partial n_2}{\partial t} &= D_n \frac{\partial^2 n_2}{\partial x^2} + P_1(S)n_1 - P_2(S)n_2 - d_n n_2 \\
 \frac{\partial m}{\partial t} &= d_n n_2 \\
 \frac{\partial S}{\partial t} &= D_s \frac{\partial^2 S}{\partial x^2} - \frac{q_t(Kn_1 + n_2)S}{S + S^*} - \frac{q_h(1 - n_t)S}{S + S^*} + Q_0 EC \\
 P_1(S) &= k_1 \exp(-k_2 S), \quad P_2(S) = k_3(1 - \tanh[(S_{\text{crit}} - S)\varepsilon])
 \end{aligned} \tag{1.1}$$

where D_s is the diffusion coefficient of the substrate, q_t is the rate of the consumption of the substrate by the tumor, K is the parameter determining the difference in the nutritive material consumption intensity between the migrating and dividing cells, S^* restricts the consumption of the nutritive material by the cells for its low concentration, q_h is the coefficient of the consumption of the substrate by the normal tissue, Q_0 is the parameter determining its supply from the vessels whose density in the tissue is given by the variable $EC(r, t)$. It is adjusted so that the constant level of nutritive

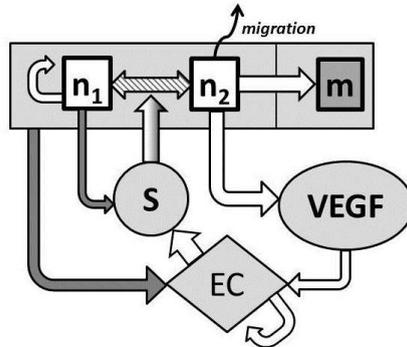


Figure 1. Block diagram of the model. White arrows indicate stimulating relations, black arrows correspond to inhibiting relations.

material $S(r, t) = 1$ is maintained in the absence of a tumor in the tissue with a pre-existing vascular system $EC_0 = 1$. It should be noted that the model uses only nonnegative parameters.

The form of the functions $P_1(S)$ and $P_2(S)$ describing the intensities of the transition from the proliferating state to the migrating one is significant. The transition intensity $P_1(S)$ was taken from [14] where it was successfully used for fitting experimental data. The parameter k_1 characterizes the maximal transition intensity, k_2 characterizes the sensitivity to a shortage of nutritive material. Unfortunately, there is no information confirming experimentally the form of the function $P_2(S)$. Therefore, we have used a smooth function close to stepwise, where S_{crit} is the concentration of the nutritive material indicating the level above which a cell can stop migration and begin division, $2k_3$ is the maximal intensity of the transition from migration to proliferation, the parameter ϵ characterizes the distinction of the function $P_2(S)$ from a stepwise one, i.e., $P_2(S) = 2k_3\Theta(S - S_{crit})$, for $\epsilon \rightarrow 0$. We have already used this form of transition function in the simulation of an invasive tumor growth subject to the dichotomy of migration and proliferation of its cells [11].

The model considers a certain averaged characteristic of the vascular system in a tissue, namely, its density. The reasons for such approach are considered in details in Discussion. We assume that a capillary network is initially present in the tissue, and this network is sufficient for its vital activity. The density of this network is taken equal to one. In this case the vascular system can become denser (angiogenesis) depending on the concentration of the vascular endothelial growth factor $V(r, t)$, and inside the

tumor the capillaries are destroyed. The distribution of VEGF in a tissue is determined by the balance of its production by the tumor cells, the diffusion, nonspecific degradation, and utilization by the endothelial cells forming the vascular system. Thus, the equations for the density of the vascular system in a tissue and for the concentration of the proangiogenic factor take the following form:

$$\begin{aligned} \frac{\partial EC}{\partial t} &= \frac{RV}{V + V^*} EC(1 - EC/EC_{\max}) - l_n EC \\ \frac{\partial V}{\partial t} &= D_V \frac{\partial^2 V}{\partial x^2} + p(fn_1 + n_2) - d_V V - \omega VEC \end{aligned} \quad (1.2)$$

where R is the maximal growth rate of the vessels, l is the degradation rate of the vascular system inside the tumor, D_V is the coefficient of the VEGF diffusion, p is the rate of production of the VEGF by the tumor cells, f is the ratio of the production rates for different types of malignant cells, d_V is the rate of nonspecific degradation, ω is the rate of VEGF utilization by the endothelial cells of the vascular network in the process of angiogenesis.

System of equations (1.1)–(1.2) obtained here allows us to simulate both the growth of the tumor depending on the supply of nutritive material through the vascular network into the tissue and the change in the vessels caused by this growth.

2. Results

It is well known that a tumor has a spherical form at the initial stage of its growth. With an increase of its radius up to several millimeters, a necrotic zone is formed in its center, and this zone contains no living cells. Since the differences in the Laplace operator for the spherically symmetric and plane cases are essential for small radii only, the use of the plane geometry for simulation of tumors with a central necrotic zone does not lead to any significant errors in the result. Therefore, we solve system of equation (1.1)–(1.2) in a one-dimensional planar domain of length $L = 2$ cm assuming that the center of the tumor lies on the left boundary and the tumor grows to the right in the direction of the normal tissue with a dense vascular network.

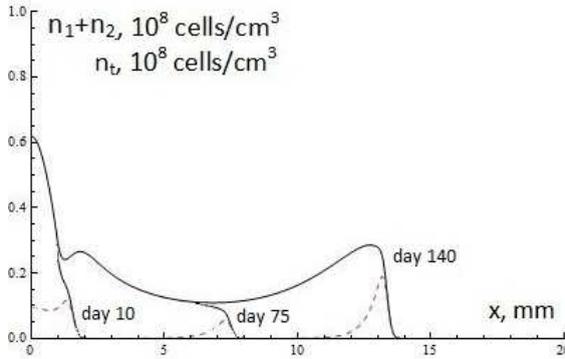


Figure 2. Distribution of densities of living $n_1 + n_2$ (dotted line) and all $n_1 + n_2 + m$ (solid line) tumor cells on the 10th, 75th, and 140th day of growth.

Thus, the boundary conditions have the form

$$\left\{ \begin{array}{l} n_{1x}(0,t) = 0 \\ n_{2x}(0,t) = 0 \\ m_x(0,t) = 0 \\ S_x(0,t) = 0 \\ EC_x(0,t) = 0 \\ V_x(0,t) = 0, \end{array} \right. \quad \left\{ \begin{array}{l} n_1(L,t) = 0 \\ n_2(L,t) = 0 \\ m(L,t) = 0 \\ S(L,t) = 1 \\ EC(L,t) = 1 \\ V_x(L,t) = 0. \end{array} \right. \quad (2.1)$$

The model has a lot of parameters whose values have been taken mainly from published data. We take Lewis lung carcinoma (LLC) as a basic type of tumor. This is a well-known intertwined tumor possessing a high metastatic potential and hence high invasion. The main kinetic parameters of this type of tumor cells were presented in [14]. The critical metabolite in that paper was presented by glucose, and not oxygen. Therefore, we also take glucose as the nutritive material in our model. This is admissible for a qualitative description, because the domains of hypoxia and glycohemia (the shortage of oxygen and glucose, respectively) in the tumor [3] practically coincide. In addition, paper [14] presents an estimate of the rate of VEGF production by tumor cells in the state of a metabolic stress. Other parameters determining the dynamics of VEGF in a tissue were taken from [13]. The greatest problems appeared in the determination of parameters for equations (1.2) for the vascular network density, because this equation describes a certain averaged characteristic. The primary estimate of the maximal growth rate R for the vascular network density was taken from [19]. The other parameters

of the model were taken from physiologically justified ranges, to reproduce the known tumor structure in the tissue.

For the sake of computation convenience, all parameters were taken dimensionless. The normalization values were the following: $t_0 = 1$ (hour) for time, $L_0 = 10^{-2}$ (cm) for length, $n_{\max} = 10^8$ (cell/ml) for the cell density, $S_0 = 1$ (mg/ml) for the glucose concentration, and $V_0 = 10^{-13}$ (mol/l) for the concentration of VEGF. As has been already said, the normal density of the vessels in the tissue was taken equal to one, i.e., $EC_0 = 1$. After removing the dimensionality, the following basic set of parameters was chosen:

$$\begin{aligned}
 L &= 200, & B &= 0.047, & d_n &= 0.01 \\
 D_n &= 0.036, & D_S &= 108, & D_V &= 21.6 \\
 k_3 &= 0.12, & S_{\text{crit}} &= 0.3, & \varepsilon &= 10 \\
 k_1 &= 0.4, & k_2 &= 19.8, & q_h &= 0.1275 \\
 q_t &= 5.1, & K &= 0.025, & S^* &= 0.02 \\
 f &= 0, & EC_{\max} &= 3, & Q_0 &= 0.125 \\
 p &= 20, & d_V &= 0.1, & w &= 1 \\
 R &= 0.075, & l &= 1, & V^* &= 0.1.
 \end{aligned} \tag{2.2}$$

Using this set of parameters, we performed a numerical study of the model. At the initial time moment $t = 0$, for the whole domain we assumed that $S(x, 0) = 1$, $EC(x, 0) = 1$, $V(x, 0) = 0$, $n_2(x, 0) = m(x, 0) = 0$, a small population of dividing cells is found only near the right boundary of the domain $n_1(x, 0) = 0.5 - 0.02x^2$ for $x \leq 5$ and $n_1(x, 0) = 0$ for $x > 5$.

Numerical solution of system of equations (1.1)–(1.2) was performed by the method of splitting with respect to physical processes. All kinetic equations were solved by the fourth-order Runge–Kutta method, the transfer equations were solved by the Crank–Nicolson scheme.

The results of model calculations are presented in Figs. 2 and 3 for the standard set of parameters. As is seen from these graphs, the results of modelling correctly represent the tumor structure after its formation, i.e., the layer of the active cells on the boundary and the necrotic zone at the center of the tumor. An increased density of the vascular network is seen on the interface of the tumor and the normal tissue, the vessels inside the tumor are practically absent.

The key question to which our investigation must give an answer is how strongly angiogenesis affects the invasive tumor growth rate. For this purpose, we varied the two parameters R , l of equation (1.2) for the vascular

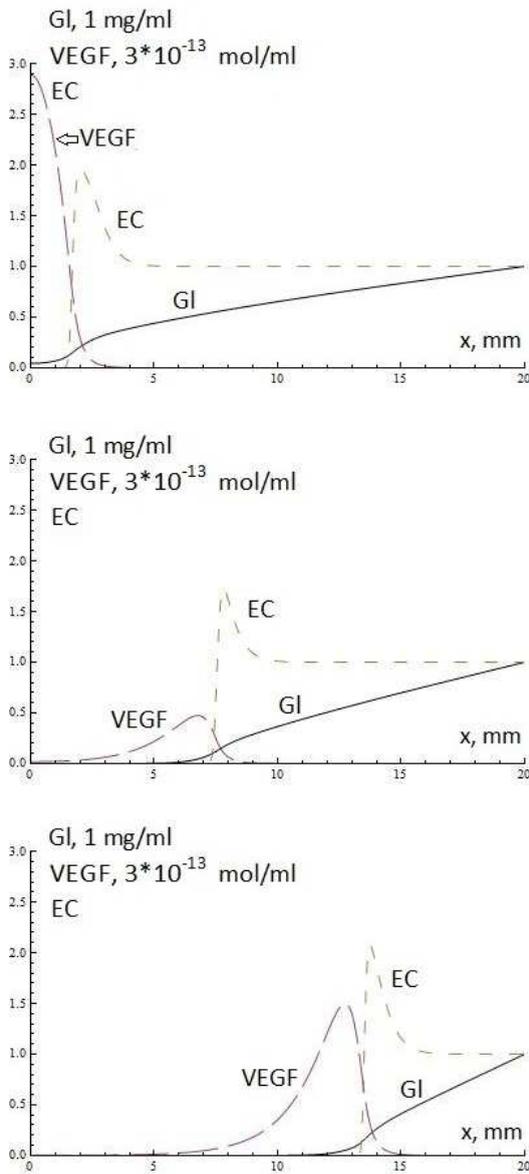


Figure 3. Distributions of glucose and VEGF concentration, and also the density of the vascular network in the tissue on the 10th (top), 75th (middle), and 140th (bottom) days of tumor growth.

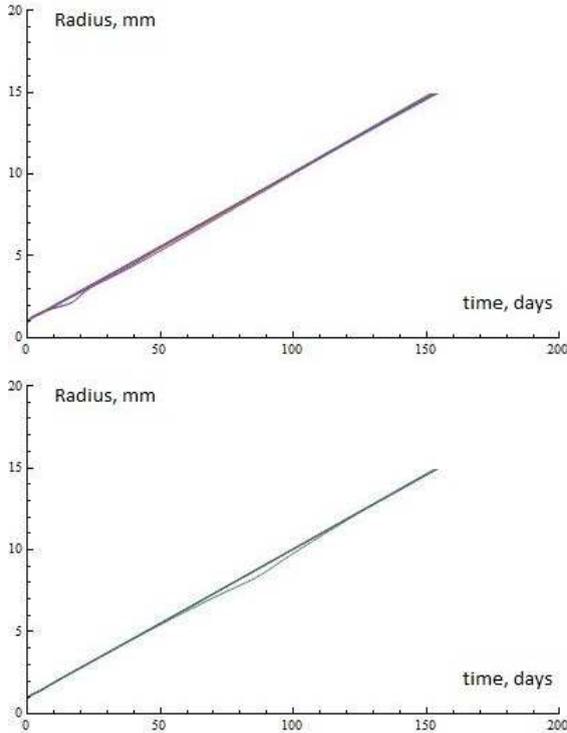


Figure 4. Tumor growth rate depending on the parameters determining the reconstruction of the vascular network, R (left), l (right). The parameters were varied alternately, $R = 0; 0.0025; 0.005; 0.0075; 0.01; 0.015$, $l = 0.1; 0.25; 0.5; 1; 2.5$, the other parameters were taken from the standard set.

network density. It should be noted that both these parameters varied within very wide ranges, i.e., $R \in [0; 0.015]$, $l \in [0.1; 2.5]$. In this case the maximal value $R = 0.015$ corresponds to the doubling of the vascular network density in 4 days (under a high concentration of VEGF), which is close to the physiological limit for the angiogenesis rate. The zero value $l = 0$ is not admissible for the rate of vascular network degradation by the tumor, because in this case some sources of nutritive materials remain inside the necrotic zone (and hence active cells remain there too), which is not observed in the experiments. Histological data show that even if a vessel remains inside a tumor, the considerable thickness of its walls hampers the supply of nutritive materials from the blood into the tissue. Figure 4 presents the dependence of the tumor radius on time for different values of R , l . The radius was chosen as the maximal value of x such that $n_1(x, t) + n_2(x, t) > 0.05$. As is seen from

the graph presented here, the invasive tumor growth rate does not depend on the parameters determining the rate of the reconstruction of the vascular network.

Conclusion

In this paper we have considered a continual multicomponent model of an invasive tumor growth taking into account angiogenesis. Choosing the type of the model, we assumed that the blood flow and hence the nutritive material transfer in the capillaries cannot be efficiently described by means of hydrodynamic equations, because the typical capillary cross-section sizes (5–10 micrometers) are three orders smaller than the sizes of tumors detected by modern diagnostic methods (about 1 cm). Moreover, there are many technical problems related to such description, in particular, a lack of data concerning the structure and properties of the capillary tree even in a normal tissue, variations of the capillary cross-section depending on blood pressure and the surrounding tissues, etc. An approach considering the density of the vascular network seems to be more promising. The main problem in this case is the correct consideration of the variation in the nutritive material supply caused by the vascular network reconstruction. A reasonable question arises: can one associate this supply only with the local density of the vascular network? The answer to this question is important for mathematical modelling of processes accompanied with angiogenesis. For our model we have considered the simplest linear relation between the vascular network density and the nutritive supply into the tissue. This can be regarded as a deliberate overestimation of this relation and, therefore, the result obtained here is even more reliable: angiogenesis does not affect the invasive tumor growth rate even under such overestimation.

The key factor determining this result is the consideration of an invasive tumor. Transforming the random mobility of the tumor cells into a dimensional variable, we get $D_n = 10^{-9}$ (cm²/s), which is a large value generally assigned to metastatic, highly invasive types of tumors, for example, to gliomas [16]. For tumors whose cell mobility is lower by an order, in addition to diffusive transfer, one should also take into account the convective flows in the tissue appearing due to active proliferation of the malignant cells [10]. Those flows depend on the total supply of nutritive material into the tumor and hence ought to have a stronger dependence on the tumor an-

giogenesis. In this case the angiogenesis may possibly stimulate the tumor growth. We are only planning such investigation.

Folkman [6] made a conjecture that angiogenesis plays a key role just for metastatic tumors, because it develops a vascular network and thus promotes the penetration of malignant cells into the circulatory system, which leads to the formation of secondary tumor focuses, i.e., metastases. However, the results of simulation have shown that angiogenesis does not influence the growth rate of a metastatic (invasive) tumor. Moreover, angiogenesis generally causes the formation of new capillaries with the diameters comparable with the size of tumor cells, which does not promote the entry of such cells into the circulatory system. All this indicates that angiogenesis is not a key process in the development of metastatic tumors. From the practical viewpoint, this means that the use of antiangiogenic therapy is inefficient for treatment of invasive (metastatic) tumors, which has been confirmed by a series of experimental studies [9].

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