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A computer algorithm describing the process of vessel formation and maturation, and its use for predicting the effects of anti-angiogenic and anti-maturation therapy on vascular tumor growth

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Abstract

We put forward an algorithm describing the three principal interconnected sub-processes that influence tumor and vasculature dynamics: (i) tumor cell proliferation (ii) angiogenesis, that is, the formation and regression of immature vessels (IV), and (iii) maturation, i.e., the formation and destabilization of mature vessels (MV). This algorithm takes account of the crucial quantitative interactions of these sub-processes, occurring across the molecular, cellular and organ levels. Implementing this complex algorithm in a computer model, one can evaluate the correlations between various factors influencing angiogenesis and their influence on tumor progression at any given moment. Moreover, the computer simulations enable analysis of the versatile effects of drugs on the growth and decay of both the tumor and the immature and mature blood vessels, as well as on the induction of an array of relevant growth factors such as angiopoietin-1 (Ang1), angiopoietin-2 (Ang2), vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). Simulation results suggest that vessel maturation and destabilization of MV drive the otherwise non-linearly growing system into a very dynamic region, having irregular, scale-invariant, fluctuations, around certain asymptotic values of all the involved quantities. Destabilization itself adequately explains the experimentally observed eventual decrease of tumor growth, with no need to implicate additional assumptions, such as a new tumor growth inhibitory, or anti-angiogenic, factors. Our results further suggest that mono-therapy alone can slow tumor growth, but is not capable of eliminating it altogether. In contrast, the combined treatment of anti-angiogenic and anti-maturation drugs causes prolonged suppression of tumor growth and a significant linear decrease in average tumor size. Laboratory experiments are warranted for validating our predictions and for providing *in vivo* evaluated parameters.

Abbreviations: Ang1 – angiopoietin 1; Ang2 – angiopoietin 2; EC – endothelial cells; EVD – effective vascular density; IV – immature vessels; MV – mature vessels; PDGF – platelet-derived growth factor; VEGF – vascular endothelial growth factor

Introduction

Growth of malignant tumors more than 1–2 mm in diameter critically depends on their neo-vascularization, which provides vital nutrients and growth factors, and also clears toxic waste products of cellular metabolism [1]. Indeed, angiogenesis – the formation of new blood vessels by budding from existing ones – has proven widespread significance in clinical oncology. Its role as a target for cancer therapy, first recognized by Folkman in 1971 [2], received wide acceptance in the early nineties following the discovery of the first specific anti-angiogenic substances by O'Reilly et al. [3, 4]. This approach seems advantageous in being universal for different types of solid tumor and in lacking major side effects.

Intensive research during the last 15 years has led to a better understanding of this complex process [5–12]. Thus, it is now understood that the genetic features of the tumor and the availability of the nutrients are the major determinants of new vasculature formation. Under conditions of nutrient deprivation tumor cells secrete stimulatory factors such as vascular endothelial growth factor (VEGF), which is a potent stimulator of endothelial cell (EC) proliferation and migration [13–20]. Consequently, additional blood vessels are formed and the signal for increased VEGF production disappears. If the basic, genetically determined VEGF

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expression is lower than a give certain survival threshold, newly formed blood vessels regress [21–23]. This negative feedback can produce successive cycles of growth and regression of blood vessels [24].

Direct *in vivo* experiments show that newly formed blood vessels are dynamic structures, continuously undergoing growth and regression [23, 25, 26]. This dynamic instability can be reversed by vessel maturation, through IV coverage by pericytes [25, 26], governed by platelet-derived growth factor (PDGF) and the angiopoietin system [23, 27–31]. Like VEGF, angiopoietin 1 (Ang1) and angiopoietin 2 (Ang2), as well as PDGF expression can be influenced both by genetic and by micro-environmental factors.

Recent vessel maturation research pinpoints the significance of the Angiopoietins/Tie-2 System [29–31]. This system includes the endothelium-specific receptor tyrosine kinase, Tie-2, its agonist, Ang1, and the natural antagonist Ang2. Ang1 promotes vessel maturation, while Ang2 antagonizes its action and can destabilize mature vessels (MV) [27, 28]. Ang1 and Ang2 can be expressed variably in human tumor cells or in EC, depending on the individual tumor type [27, 28, 32–34].

The dynamics of angiogenesis must be better understood in order to establish successful anti-angiogenic treatment protocols [35]. However, as was illustrated above, the comprehensive angiogenesis dynamics are too intricate to be captured by intuition alone, since they involve several interacting oscillatory processes, which operate on several time scales. Notably, these oscillations involve the total perfused vasculature (to be denoted effective vascular density, EVD), the generation of immature vessels (IV) and their regression, and the maturation and destabilization of MV. By mathematically modeling each of the crucial dynamics, subsequently to be calculated numerically, one can test the potential effects of various drugs and drug schedules on the system.

Vascular tumor growth, including dynamics of both vasculature and malignant cells, has been described in mathematical models by Hahnfeldt et al. [36] and Bellomo, Preziosi et al. [37, 38]. Hahnfeldt et al. [36] propose a macroscopic model, assuming logistic tumor growth. However this model does not take into account vessel maturation nor does it allow for the nutrient-dependent secretion of pro-angiogenic factors. A model by Preziosi and co-authors [37, 38] describes the vascular tumor system on three scales: molecules, cells, and macroscopic entities (such as tumor volume). This model is much more detailed than the previous one [36]; however, it also fails to include maturation of new blood vessels.

In this work we analyze the complex system of interactive hierarchical processes involved in angiogenesis, by implementing the various empirically observed angiogenesis-related interactions in a detailed algorithm of vascular tumor growth. The algorithm is novel, principally because it takes account of vessel maturation and of destabilization of MV. It makes new assumptions about the relationships and the driving forces in this system and examines their qualitative contribution to experimentally observed phenomena. As it accounts for the specific interactions between tumor dynamics, nutrient-dependent production of angiogenic factors, vascular growth and regression and vessel maturation, one can use this algorithm for evaluating novel anti-angiogenic therapies.

In the work presented here we theoretically investigate the properties of the new algorithm by analyzing potential tumor and vasculature dynamics under different sets of parameters. In order to explore the potential use of the algorithm for determining the success of putative clinical anti-angiogenesis treatments, we model the influence of angiogenesis-directed treatments, as well as combined therapeutic intervention with both anti-angiogenic and anti-maturation drugs. Lacking estimations of real-life angiogenesis-related parameter values we use here arbitrary dimensionless units for all model parameters. In future work, the present algorithm will be validated using clinical trial results. To this end we will first attempt to estimate the human angiogenesis parameters, which are clinically relevant *in vivo*. Subsequently, a detailed parameter variability analysis will be conducted, so that the quantitative significance of our results can be evaluated.

Methods

Description of the angiogenesis algorithm

Our angiogenesis algorithm makes several new assumptions about the relationships between the various driving forces, which operate simultaneously during neo-vascularization. Six major processes are described in three interconnected modules: (i) tumor cell proliferation and death; (ii) IV formation and regression; (iii) IV maturation and mature vessel destabilization (Figure 1). Each module operates on three scales: molecular, cellular and macroscopic, namely, vessel densities and tumor volume.

The tumor module consists of tumor cell proliferation and death, further subdividing into (i) time-invariant, cell type-specific, genetically determined block, and (ii) time-variant, nutrient-dependent block. Nutrient-dependent proliferation and death rates are proportional to the EVD, the former directly, the latter inversely [39, 40]. Two additional quantities are calculated in this module, namely VEGF and PDGF production. They are inversely related to EVD so that increasing nutrient depletion results in increasing secretion of pro-angiogenic factors [7–9]. The tumor growth module interacts with the angiogenesis and the maturation modules *via* the relevant regulatory proteins.

In the angiogenesis module we calculate IV volume. IV volume increases proportionally to the VEGF concentration if the VEGF is above a given threshold

level, and regresses if the VEGF is below a given, possibly different, threshold level. The latter threshold is generally referred to as 'survival level' [21–24].

In the maturation module we calculate MV volume according to pericyte concentration [12] and according to the Ang1/Ang2 ratio [41]. Pericytes proliferate proportionally to PDGF concentration [25–26]. Ang1 and Ang2 are continuously secreted by tumor cells and IV, respectively [27, 28, 32–34]. Additionally, Ang2 can be secreted by tumor cells, if the latter are nutrient-depleted [28]. We assume that maturation of IV occurs if the pericyte concentration and the Ang1/Ang2 ratio are above their respective threshold levels, whereas below these thresholds IV do not undergo maturation and MV undergo destabilization and become immature [29–33].

Analysis and numerical simulations

The above algorithm is precisely described mathematically by a large set of formulae, which account for each and every interaction (i.e., arrow) in Figure 1. The full model is much too complex to be tractable to mathematical analysis, and is beyond the scope of this paper. A simpler version, in the form of several ODE systems, encoding the most essential assumptions about the complex hierarchical interactive processes of tumor neo-vascularization, has been analyzed elsewhere [42, Daugulis et al., submitted] (see discussion below). In the work presented here, the comprehensive algorithm has been studied in recursive numerical simulations, which have been performed using Excel software on a PC computer. Aiming at this stage to analyze the qualitative behavior of the model we used arbitrary dimensionless units for all model parameters [43]. Initial conditions were 100 tumor units and one unit vascular density. Calculation step duration is equivalent to the generation time of tumor cells, that is, to one cell cycle.

At every time step the model calculates the tumor size, which is determined as a function of tumor cell number, the number of free EC and pericytes, the concentrations of the regulatory factors (VEGF, PDGF, Ang1 and Ang2), and the volume of both IV and MV.

In addition, the density of both IV and MV, that is, the volumes of corresponding vessels divided by tumor size, are calculated and added up to produce an EVD; EVD is defined as the sum of IV density and of MV

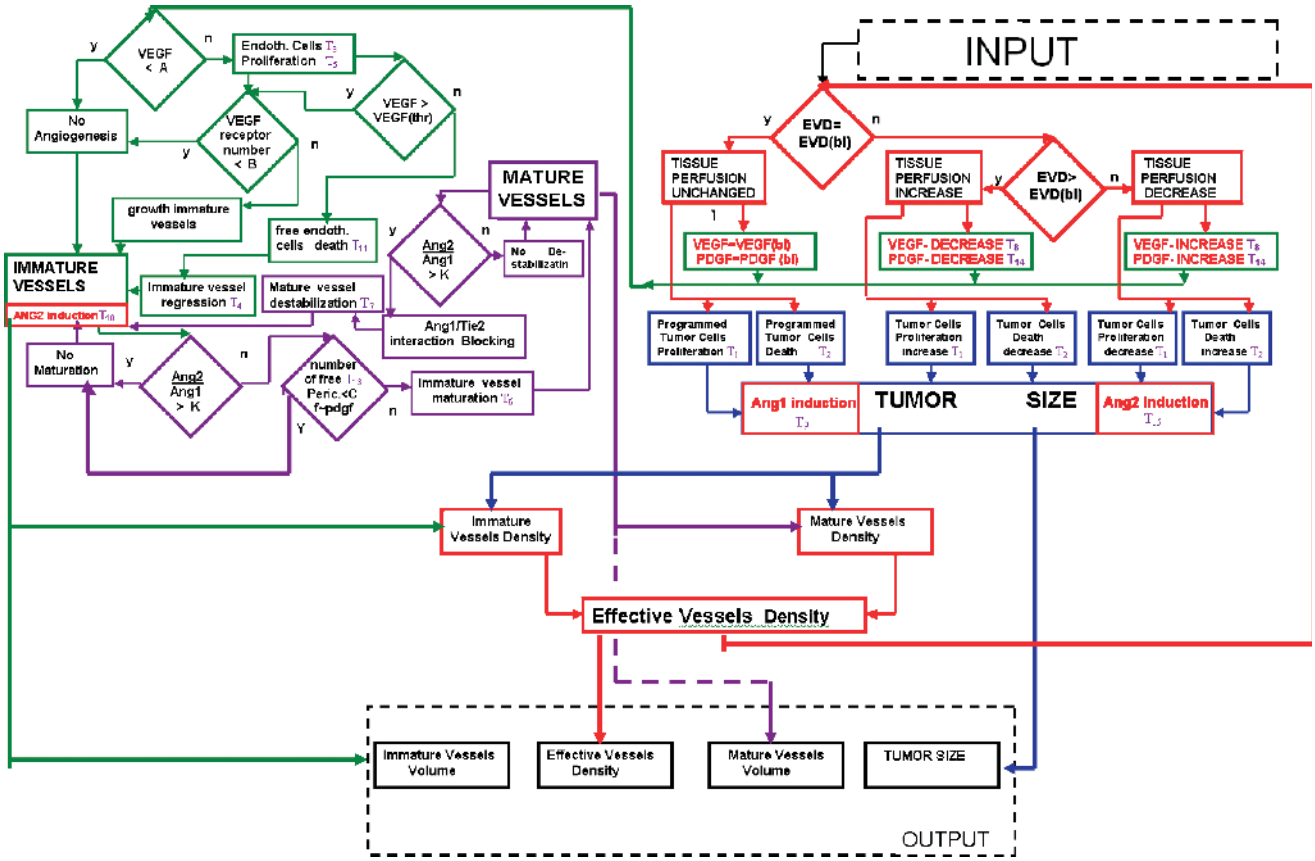

Figure 1. Algorithm describing the principal interactions affecting vascular tumor growth. The algorithm describes the interrelationships between tumor growth, the formation of new vessels (angiogenesis) and the maturation of the newly formed vessels. The interactions considered occur across three organization levels: molecular, cellular, and organic level. The arrows indicate the specific module interaction; the boxes indicate the moment of specific sub-process calculations; the diamonds indicate the conditions, which determine the direction of processes. Green – the angiogenesis module; purple – the maturation module; blue – changes in tumor size; red – changes of the effective vessel density. EVD_{bl} – The value for which the system is in steady-state; $VEGF_{bl}$ – VEGF secretion level at the steady-state of the system; $VEGF_{Thr}$ – VEGF concentration below which EC, both in the free state as well as when incorporated into immature blood vessels, are subject to apoptosis; $PDGF_{bl}$ – PDGF secretion level at the steady-state of the system.

density. Note that IV and MV do not have the same efficiency at conducting flow and perfusing tissue, due to the morphological and physiological differences between them. For this reason, the perfusion by each type of vessel at every moment of the process is calculated separately with vessel-specific perfusion parameters. However, for simplicity of the present work, perfusion efficiency is assumed to be the same in IV and MV. Obviously, this constraint will be relaxed when real-life angiogenesis is evaluated. The model assumes several threshold- and ratio-dependent effects of regulatory factors, as follows:

- a threshold of VEGF concentration above which EC proliferation takes place;
- a threshold of VEGF concentration under which free EC, as well as those incorporated into immature blood vessels, undergo apoptosis;
- a threshold concentration of free pericytes above which IV can mature;
- Ang1/Ang2 ratio above which IV mature and below which MV are destabilized.

Model variables are calculated at each time step, serving as input values for the next time step. Concurrently, they are compared with threshold levels, and new values of model variables are calculated according to the arrows on the scheme (Figure 1).

For simplicity, all the thresholds in the model assume unity weights to all the related quantities. However, in further work, when real-life situations are analyzed, the weights will be re-evaluated and their values will be adjusted for each concrete case.

Results

Computer simulations of the tumor growth model are represented as time series of the measured quantities: the EVD, the tumor size, the concentrations of VEGF, PDGF, Ang1 and Ang2, Ang1/Ang2 ratio, number of free pericytes and the IV and MV volume. Time is measured in cell cycles, while values on the *y*-axes in all graphs are expressed in arbitrary dimensionless units.

In order to investigate the relative contribution of different dynamic sub-processes to the overall tumor growth we performed several 'knock-out' simulation experiments, simply by taking the relevant reaction rates as null. We started by 'knocking-out' many sub-processes and, by activating one function at a time, we gradually increased the system's complexity. In this way we could examine the relative contribution of each function to the overall process. Results are presented in Figures 2–4.

Figure 2.1 shows a simulation experiment, in which all features of vasculature dynamics are knocked-out, except for the formation of new vessels. At any moment in this simulation, and in all subsequent simulations, we calculate vascular densities (a) vascular volume (b) Ang1 and Ang2 concentrations (c) and tumor size (d). As

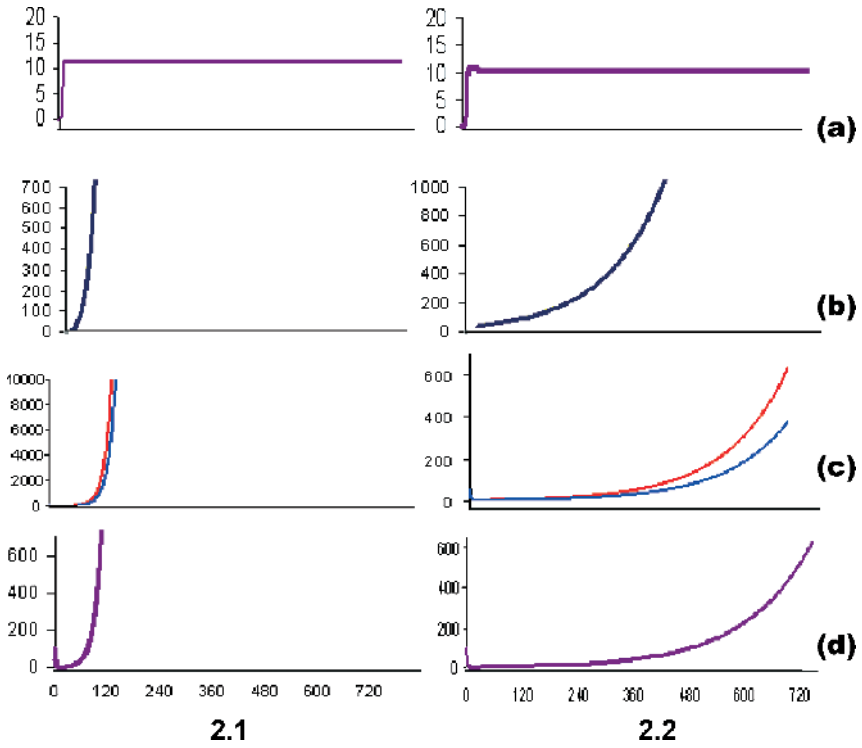

Figure 2. New vessel formation and IV regression. Simulation results of the model embedded in the algorithm presented in Figure 1. EC proliferation coefficient is 108. The coefficients of IV maturation and MV destabilization are both equal to zero. Regression coefficient is 0.0 in 2.1 and 0.3 in 2.2. (a) Vascular density plotted as a function of time; (b) vascular volume plotted as a function of time; (c) Ang1 (blue) and Ang2 (red) concentration plotted as a function of time; (d) tumor size plotted as a function of time.

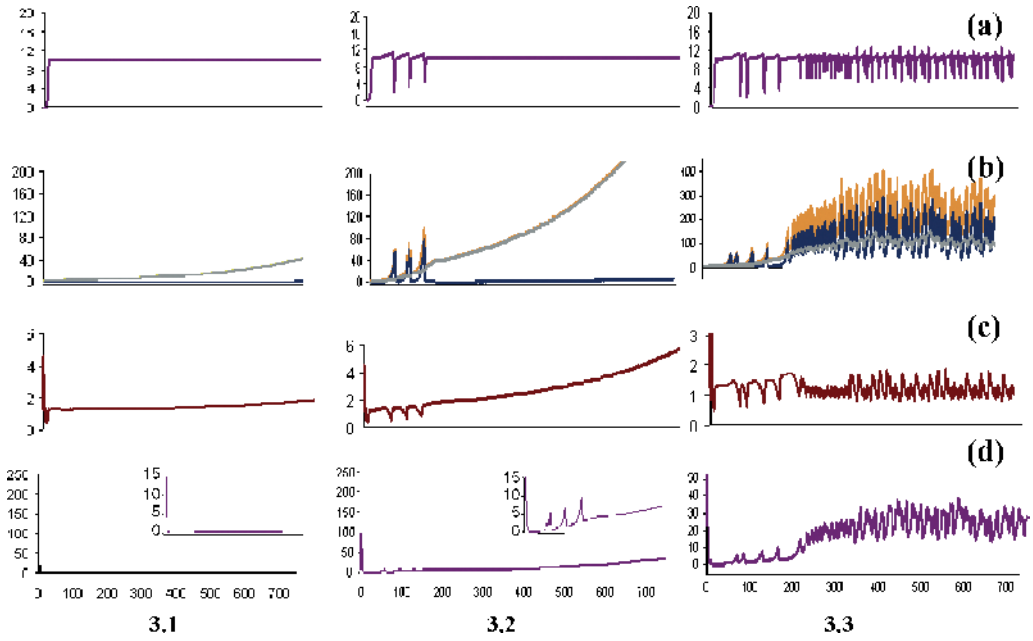

Figure 3. Effects of IV maturation and MV destabilization on tumor, vascular and growth factors dynamics. Simulation results of the model embedded in the algorithm presented in Figure 1. 3.1: Maturation coefficient is 0.58, destabilization coefficient is 0; 3.2: maturation coefficient is 0.57, destabilization coefficient is 0; 3.3: maturation coefficient is 0.57, destabilization coefficient is 0.2. All other parameters are equal to those in Figure 2. (a) Vascular density plotted as a function of time; (b) vascular volume plotted as a function of time (MV – green; immature – blue); (c) Ang1/Ang2 ratio plotted as a function of time; (d) tumor size plotted as a function of time.

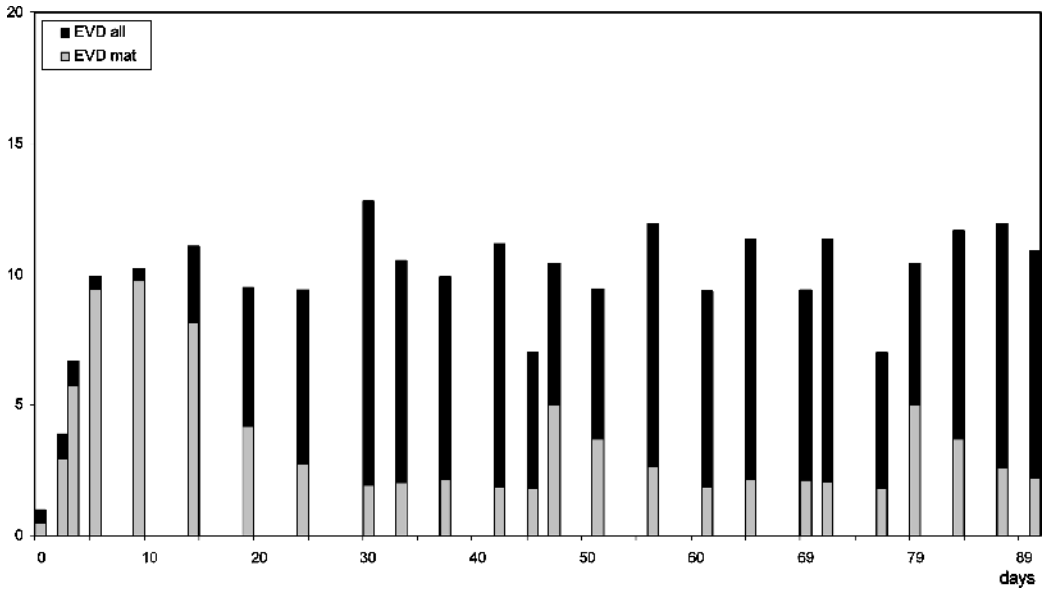

Figure 4. Simulation results showing fluctuation over time total (black) and mature (gray) vascular densities during the initial (dormant) phase of tumor growth. All parameters are the same as in Figure 3.3.

expected, one can see in Figure 2.1 that when no IV regression is allowed for, and vessel maturation is knocked-out as well, tumor and vascular volumes follow simple exponential growth curves, so that the overall dynamics is relatively simple. The addition of vascular regression (Figure 2.2) slows the growth rates, but the general form of the curves does not change, despite the fact that, in this simulation, the maximal vascular regression rate is 8 times greater than the maximal vascular proliferation rate. In both cases the increase in vascular volume closely follows the increase in tumor volume. Consequently, vascular density (Figure 2.1) remains constant after an initial rapid increase. This stable value of vascular density is just a little above the effective vessel density of a steady-state tumor.

Vascular maturation is introduced in Figure 3.1, where destabilization of MV is still 'knocked-out' and the maximal regression rate is just above the maximal EC proliferation rate. One can see in this figure that vessel maturation significantly slows tumor and vascular

growth. A decrease in maturation rate (Figure 3.2) facilitates tumor growth, and also results in an early burst of fluctuations.

The introduction of MV destabilization increases tumor and vascular growth (Figure 3.3(d)). Indeed, it can be expected that the recurrent flow from the mature to IV compartments may have this effect, as IV volume is expected to increase faster than MV volume, and perfusion rate is taken to be similar in both (see above). However, the most dramatic phenomenon resulting from the introduction of destabilization in this parameter range is the shift of the system from transient instability to harsh instability, where all model variables undergo erratic fluctuations on several scales around certain asymptotic values (Figure 3.3).

In Figure 4 we examine more closely the simulated vascular dynamics during the initial phase of tumor growth, in which tumor volume does not increase significantly during relatively long periods of time (simulating dormancy in real-life tumors). The simulations, using the same parameters as in Figure 3.3, are characterized by fluctuations of total, as well as mature, vascular densities. This result is in remarkable qualitative agreement with the published results of MRI measurements of tumor vascularity in mice [24].

At the present stage, we can remark on the qualitative similarity between the theoretical and the experimental angiogenesis patterns. However, since values of the relevant empirical angiogenic parameters are not yet available, we cannot determine whether the theoretical parameter ranges being studied are of relevance in real life. Following the substitution of empirical parameter values into the model and after performing appropriate adjustments for the remaining parameters, the quantitative fit of the model predictions to the experimental results can be estimated (Merbl et al., in preparation).

If, indeed, the above remarkable non-linearity, apparent in our simulations, characterizes real-life cancers, it may have significant implications for anti-angiogenic drug therapy, possibly also resolving some of its current elusiveness [35]. Below we simulate two examples of 'prototypical' anti-angiogenic and anti-maturation therapy.

Simulating the effects of anti-angiogenesis and anti-maturation therapies

Figures 5.1–5.3 show simulation results of the continuous administration of two different hypothetical drugs affecting vascular dynamics, namely VEGF-production inhibitor (drug A) and Ang1 production inhibitor (drug B). In order to analyze the potential effects of the initial characteristics of tumor vasculature on therapy success, we apply the drugs under three different sets of initial conditions. In all sets we assume the same initial tumor size, the same reaction coefficients and the same initial total, immature and mature, vascular volume. However, the percentage of IV varies, being 50% in Figure 5.1, 95% in Figure 5.2, and 5% in Figure 5.3. For each set we apply no drug (column 1), drug A (column 2), drug B (column 3), or both drugs (column 4). We show the resulting time series of vascular densities (row a), Ang1 and Ang2 concentrations (row b), and tumor size (row c).

As can be seen from Figure 5, mono-therapy by either one of the two drugs slows tumor growth, but is incapable of eliminating it. Rather, tumor size continues to increase non-linearly, even under increased drug doses and a prolonged treatment period. As can be expected, the relative deceleration in tumor growth, caused by the particular therapy, is a function of the relative proportion of the IV/MV volume: anti-VEGF therapy being more efficient when the proportion of IV is relatively large (Figures 5.1–5.3, column 2). In contrast, the overall dynamics of untreated tumor growth (column 1) or anti-Ang1 treatment efficacy (Figures 5.1–5.3, column 3) were not significantly affected by initial vasculature properties.

Unlike mono-therapy, combinational treatment of the two drugs causes prolonged suppression of tumor growth and a significant linear decrease in average tumor size (Figures 5.1–5.3, column 4). Note that this general result is not dependent on initial conditions.

Discussion

In the last decade neo-vascularization and vascular dynamics have been recognized as important phenomena of cancer development and progression. Other types of neo-vascularization have also been described, notably vascular co-option, vasculogenic mimicry and adult vasculogenesis from circulating EC. Nevertheless, angiogenesis (formation of endothelium-lined new blood vessels from existing normal vasculature) is considered as the main type of new blood vessel formation in tumors [45–48].

Due to its universality and lack of side-effects, anti-angiogenic treatment of cancer became a very attractive possibility. This concept is part of a wider approach – 'constraint-based paradigm' [44]. It suggests that instead of a detailed comprehensive description of a complex heterogeneous phenomenon (such as malignant tumor) one should focus analysis on a small number of relatively stable, universal features that function as 'bottlenecks' in the general process. Angiogenesis can be seen as such a 'bottleneck' in cancer progression, since macroscopic tumors depend on blood flow for nutrient and oxygen delivery. In an adult organism proliferating EC are limited to the female reproductive system, wound healing and tumors, therefore, anti-angiogenic drugs are expected to be highly selective and relatively non-toxic.

Extensive research of tumor angiogenesis-revealed highly complex spatially heterogeneous processes, involving multiple interactions between at least three types of cells (endothelium, pericytes and tumor cells), the extracellular matrix, and many pro- and anti-angiogenic substances. This complexity renders the intuitive

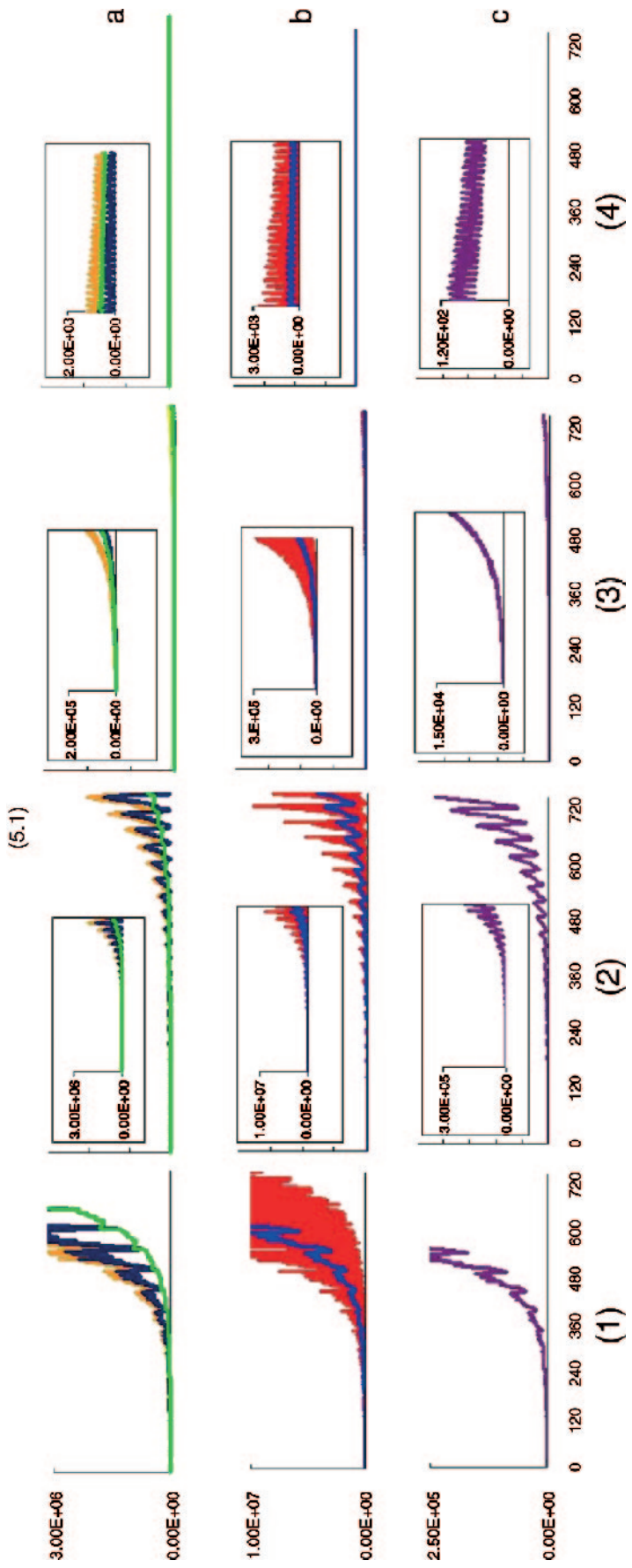

Figure 5.1. Effect of combination of anti-angiogenic and anti-maturation therapies; Simulation results of the model embedded in the algorithm presented in Figure 1. Tumor size (Ntum) is equal to 100 units. IV volume is 500 units and MV volume is 500 units. All other parameters are equal to those in Figure 2. (a) Vascular volume (MV – green; immature – blue; total – orange); (b) Ang1 (blue) and Ang2 (red) concentration; (c) tumor size. (1) Tumor with no therapy; (2) tumor following anti-VEGF therapy; (3) tumor following anti-Ang1 therapy; (4) tumor following a combined anti-VEGF and anti-Ang1 therapy.

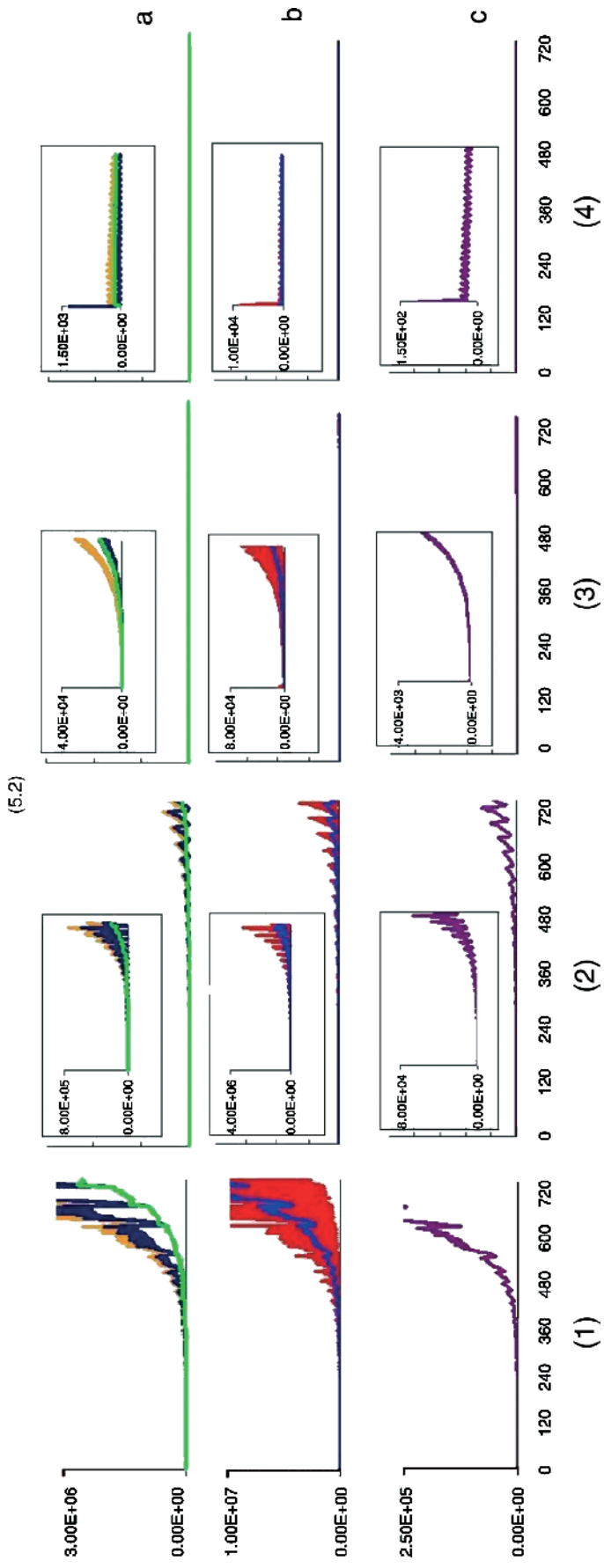

Figure 5.2. Effect of combination of anti-angiogenic and anti-maturation therapies; IV volume is 950 units and MV volume is 50 units. For all other parameters see Figure 5.1. Note differences in scales compared with Figure 5.1.

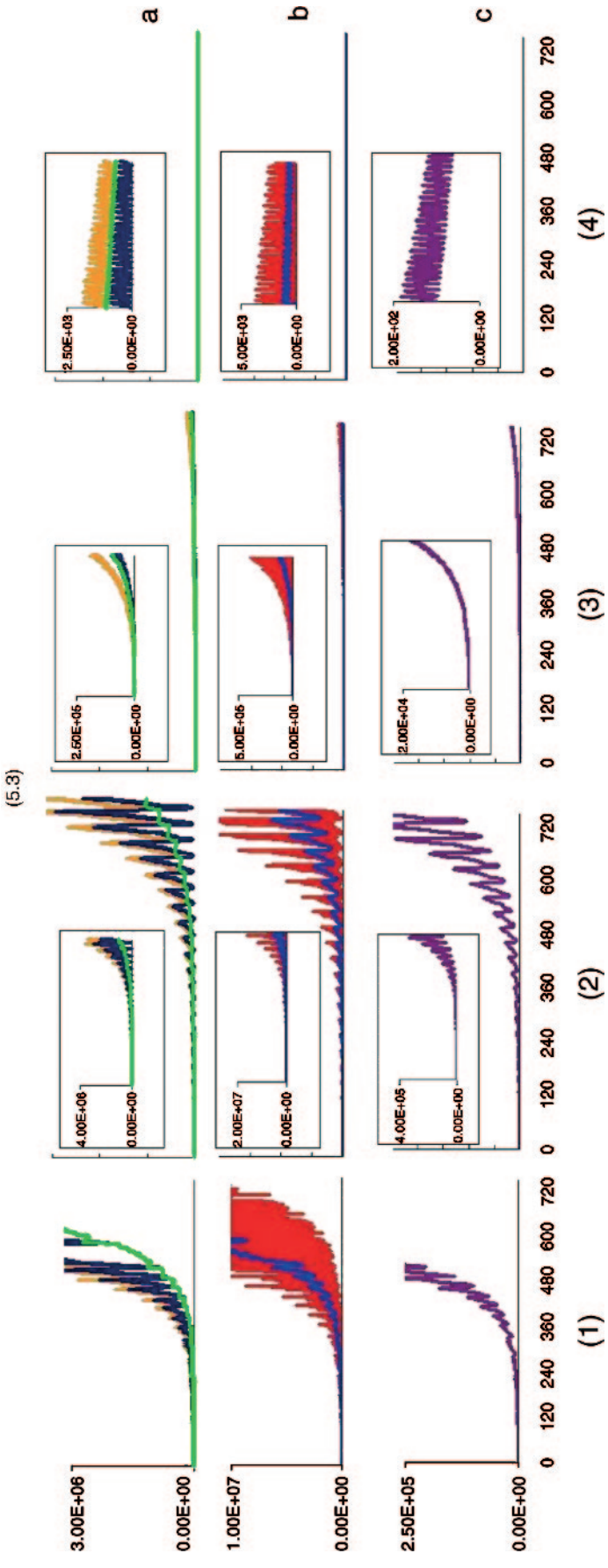

Figure 5.3. Effect of combination of anti-angiogenic and anti-maturation therapies; IV volume is 50 units and MV volume is 950 units. For all other parameters see Figure 5.1. Note differences in scales compared with Figure 5.1.

predictions of *in vivo* tumor angiogenesis dynamics very difficult. It is even more difficult to propose optimal administration schedules of anti-angiogenic drugs such as mono-therapy or schedules in combination with 'conventional' cytotoxic chemotherapy, which is recognized as a major problem of ongoing clinical trials of these drugs [35].

Mathematical modeling of vascular tumor growth can help in deciphering the complex interrelationships between angiogenesis dynamics and tumor progression. In particular, it can be used for simulating the effects of different therapeutic interventions, so that only the most essential costly and time-consuming *in vivo* experiments are conducted for prospectively validating the theoretical predictions.

In the present paper we introduced a new model of vascular tumor growth, which takes into account maturation of tumor vasculature. The model is written in the form of an algorithm, describing the numerous relationships between molecular, cellular and multicellular structures, which interactively influence tumor growth dynamics. The algorithm also takes into account the temporal parameters, which characterize reaction rates of the elements of the angiogenesis process.

This algorithm was implemented in a computer model for analyzing the influence of each of the model's subprocesses on the overall dynamics. This enabled us to analyze the effects of putative drugs on the growth and decay of the tumor, on the immature and mature blood vessels, and on the induction of an array of relevant growth factors such as Ang1, Ang2, VEGF and PDGF.

As discussed earlier, the current version of the model is relatively complex and, hence, rather difficult to analyze. Nevertheless, even in its present form the model makes several simplifications, which can be relaxed in the future. Three of those are mentioned hereafter.

First, we introduced only one angiogenic factor – VEGF. This factor was singled out as it is a major tumor pro-angiogenic substance, which serves as a target for many, currently developed, anti-angiogenic drugs. Additional angiogenic factors can be supplemented to the model, once their clinical significance is recognized.

Second, natural anti-angiogenic substances were not included in the model, since their influence is expected to be much less significant than that of the externally introduced inhibitor, the drug. Consequently, our results can be seen as applicable to aggressive tumors having low sensitivity to natural anti-angiogenic factors.

Third, since our main concern in this work is to investigate the effects of growth factor modulations on tumor progression, and those occur on a relatively fast time-scale, we assume in the present analysis that the genetically determined parameters are constant. This assumption can be relaxed in future work.

In order to examine the influence of vascular maturation on tumor and vasculature dynamics we performed a series of simulation 'knock-out' experiments in which different angiogenesis sub-processes were suppressed, by nullifying the respective rate coefficients, and the resulting dynamics were followed.

Results suggest that when angiogenesis occurs, but no vessel maturation is allowed for, the resulting system dynamics show a trend of monotonic increase of the relevant processes with tumor and vessel volumes growing exponentially. As may be expected, IV regression slows down the growth process, but does not change this general trend: tumor and vessel volumes still increase nonlinearly but the rate of increase is somewhat lower. When vessel maturation is allowed for, the general dynamics is altered and oscillatory processes in tumor and vessel volume growth begin to take effect. The magnitude of the oscillations and their time span depend much on the reaction rates. Figures 3.1–3.2 are similar in all parameter values, except for the maturation coefficient, which is marginally lower in the latter. Note that a decreasing maturation coefficient results in increased tumor growth. This is a direct result of our assumption that the IV growth rate is faster than the MV growth rate [23]. Interestingly, one can observe here that a small reduction in maturation rate drives the system into a region of dynamic instability, where all model variables undergo several significant fluctuations, followed by smooth growth. Most importantly, comparing Figures 3.1–3.2 one notes that a very small increase in maturation rate can result in the dramatic inhibition of tumor and vascular growth.

One can see in Figure 3.3 that under the same maturation rate as in Figure 3.2, but allowing for some destabilization of MV the system enters into a dynamic region, with all the processes involved having very vigorous and irregular fluctuations. As appears in Figure 3.3 it is micro-vessel destabilization, which can explain the decrease of tumor growth rate over time, as observed experimentally, e.g., by Norton [44]. Our model, relying exclusively on the dynamics of tumor maturation (Figure 3), can account for this phenomenon, having no need to introduce additional assumptions, such as new tumor growth inhibitory factors [38] or anti-angiogenic factors [36].

We were able to retrieve patterns of fluctuations in tumor vasculature, as appearing in magnetic resonance imaging (MRI) *in vivo* measurements in xenograft animal models ([24], Merbl et al., in preparation). Mathematical analyzes of several simpler versions of the model were performed in order to determine what the forces were underlying the oscillations, which characterize both the simulations of the present algorithm and the experimental results [42]. These analyses point out that oscillations of tumor growth and vascular densities, observed in our work as well as in experimental research, result from inherent time delays in the studied system.

The main conclusion of these analyses is that tumor vasculature dynamics is much too complex to allow any intuitive conclusions about their behavior to be drawn. The prediction ability is rendered even less intuitive when considering therapeutic intervention in this sys-

tem. How any externally imposed perturbation to the system will affect these highly nonlinear dynamics cannot be predicted without a numerical tool for mimicking these dynamics.

Using our vascular tumor growth model for simulating anti-angiogenic treatments, we analyzed the effects of two types of vasculature-directed treatments; the first hypothetical drug was VEGF production inhibitor, and the second – an Ang1 production inhibitor. Each drug was tested as a mono-therapy, as well as in combination under various sets of initial conditions.

Our simulation results, presented in Figure 5, show that mono-therapy alone can slow tumor progression, but fails to eliminate the tumor. This result corresponds well with recent reports of the failure of single anti-angiogenic agents in clinical trials [49]. In contrast, a combination of anti-angiogenic and anti-maturation treatments has a strong synergistic effect: tumor reduction is characterized by a prolonged oscillatory regression, with a constant average gradient, depending on the level of tumor vascularity maturation. The relative efficacy of anti-VEGF mono-therapy strongly depends on the level of vessel maturation at the onset of treatment, while efficacy of anti-maturation and combined therapies was independent of initial maturation conditions.

Our model qualitatively retrieves the prominent features of tumor angiogenesis dynamics, and points out the significant effect of vascular maturation and its dynamics on tumor growth. Quantitative predictions will hopefully follow, once its arbitrary parameter values are replaced by empirical estimations. On the basis of our results we hypothesize that combinational anti-vasculature/anti-maturation treatment may be superior to anti-angiogenic mono-therapy. This hypothesis can be further checked *in vivo*. Collaboration with experimental groups is warranted for retrieving relevant realistic values of dynamic angiogenesis parameters. Upon realistic parameters being evaluated in laboratory experiments, the current algorithm will be employed for quantitative analysis of different anti-angiogenic treatment schedules, as well as for analysis of the effects of combination therapy using anti-angiogenic and cytotoxic drugs.

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