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A HYBRID THREE-SCALE MODEL OF TUMOR GROWTH

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Abstract

Cancer results from a complex interplay of different biological, chemical, and physical phenomena that span a wide range of time and length scales. Computational modeling may help to unfold the role of multiple evolving factors that exist and interact in the tumor microenvironment. Understanding these complex multiscale interactions is a crucial step towards predicting cancer growth and in developing effective therapies.

We integrate different modeling approaches in a multiscale, avascular, hybrid tumor growth model encompassing tissue, cell, and sub-cell scales. At the tissue level, we consider the dispersion of nutrients and growth factors in the tumor microenvironment, which are modeled through reaction-diffusion equations. At the cell level, we use an agent based model (ABM) to describe normal and tumor cell dynamics, with normal cells kept in homeostasis and cancer cells differentiated apoptotic, hypoxic, and necrotic states. Cell movement is driven by the balance of a variety of forces according to Newton's second law, including those related to growth-induced stresses. Phenotypic transitions are defined by specific rule of behaviors that depend on microenvironment stimuli. We integrate in each cell/agent a branch of the epidermal growth factor receptor (EGFR) pathway. This pathway is modeled by a system of coupled nonlinear differential equations involving the mass laws of 20 molecules. The rates of change in the concentration of some key molecules trigger proliferation or migration advantage response. The bridge between cell and tissue scales is built through the reaction and source terms of the partial differential equations.

Our hybrid model is built in a modular way, enabling the investigation of the role of different mechanisms at multiple scales on tumor progression. This strategy allows representing both the collective behavior due to cell assembly as well as microscopic intracellular phenomena described by signal transduction pathways. Here, we investigate the impact of some mechanisms associated with sustained proliferation on cancer progression. Specifically, we focus on the intracellular

proliferation/migration-advantage-response driven by the EGFR pathway and on proliferation inhibition due to accumulation of growth-induced stresses. Simulations demonstrate that the model can adequately describe some complex mechanisms of tumor dynamics, including growth arrest in avascular tumors. Both the sub-cell model and growth-induced stresses give rise to heterogeneity in the tumor expansion and a rich variety of tumor behaviors.

Keywords

Hybrid multiscale model; Cell-agent based model; Signaling pathway

1. Introduction

Cancer is a complex biological multiscale phenomena, consisted of multiple inter-acting phenomena that are usually nonlinear and subject to different levels of regulation. A comprehensive understanding of cancer evolution and control requires the description and integration of events at multiple spatial scales, including the tissue, cellular, and molecular levels. The events are often embodied in intricate sequences of regulatory inter- and intracellular signaling pathways which are initiated through extracellular biochemical and biophysical cues from the microenvironment. These interconnected phenomena can only be captured by using multiscale models that integrate quantitative information at a variety of time and length scales^{22,26,41,46}.

There is now an established literature on mathematical and computational models for representing the hallmarks of cancer^{5,14,20,33}. In general, the modeling approach depends on the desired mechanisms or processes and the scales at which they take place. Most models of tumor growth that consider spatial heterogeneity are continuum cell population or discrete cell models². Continuum approach captures the average of the global cell population behavior while the discrete approach allows the tracking of individual cell dynamics. There have been many hybrid models of tumor growth which integrate both continuum and discrete approaches^{7,11,33,41}. Benefits of hybrid models include, for example, the ability to model the diffusion of nutrients and growth factors at the tissue level using partial differential equations while cell heterogeneity may be tracked through an agent based or cellular automata model^{1,9,31,35}. Cellular automata are discrete models consisting of a large number of individuals that interact with each other through well-defined rules. For the specific case of modeling cancer, the individuals are normal or tumor cells, arranged in a regular spatial grid, and each site of the grid is associated with a specific cell. The neighborhood of each cell is defined *a priori*, according to a uniform pattern⁴³. In uniform cellular automata models (CAM), interactions between neighboring cells occur synchronously, from common transition rules^{31,32}. On the other hand, agent-based models (ABM) are similar to CAM, but more flexible. In the literature, it is common to differentiate them by two main properties, determined by the mobility and the type of agent. An ABM is not restricted to just identical individuals, and there may be more than one type of individual in space. In addition, the individuals (or agents) of an ABM are not necessarily dependent on a network. Thus, the modeling of the interactions is much more versatile; in particular, it is not restricted to local interactions. From a practical perspective, ABM presents greater

flexibility in modeling cellular mechanisms. For example, because the ABM has no grid dependency, the positions of daughter cells in a mitosis and cell movement are not biased by specific routes due to lattice patterns, and can be better represented. Additionally, ABM allows for explicitly defining different interactions between agents so that there is no need to assign a single set of rules for all agents. Using this kind of approach for describing tumor cell behavior, Macklin et al.^{27,28,29} has improved the multi-cellular growth model presented in Ref. 12, developing the first patient-calibrated model of ductal carcinoma *in situ*.

The fundamental advantage of combining continuum and discrete modeling approaches is the ability to span multiscale phenomenon. Coupling signal-transduction pathways to an agent based model at the cellular scale which is then coupled to reaction-diffusion equations at the tissue scale provides new avenues for investigating the complex interplay of events occurring at tissue, cellular and sub-cellular scales. Desboeick and coworkers^{3,10,42} provided initial investigations in this direction. In particular, they developed classes of hybrid models integrating the epidermal growth factor and/or transforming growth factor α signaling pathways to investigate non-small cell lung cancer and brain tumors. However, these efforts do not explicitly account for cell interactions with the microenvironment and with other cells, or consider microenvironment heterogeneity.

In this contribution, we develop a complete hybrid framework to address significant physical and biological mechanisms of tumor progression acting from sub-cell to tissue scales. We develop consistent mathematical and computational approaches to extend the model developed in Ref. 29. In particular, we consider masses of tumors growing within healthy tissue and we model the effects of compressive stresses that accumulate within the tumor due to growth. We integrate the intracellular EGFR signaling pathway developed in Ref. 42, which allows us to investigate the effects of the downstream regulatory cell responses on tumor progression due to extracellular stimuli. We assume that individual cells may acquire proliferation or migration advantage, and we consider that haptotaxis drives migratory cells. The integration of these processes are able to describe a rich variety of avascular tumor dynamics. The developed multiscale platform is modular, allowing for the easy exchange or integration of new features.

The remainder of this manuscript is organized as follows. Section 2 describes the main biological assumptions on which the hybrid framework is built. We then present the tissue, cellular, and intracellular models and how information is translated between these three scales. In Section 3 we present a variety of simulations to highlight the main new features of the proposed framework. Our concluding remarks and perspective of future developments are presented in Section 4.

2. Model development

Our model captures key feature of several of the hallmarks of cancer^{20,21} including the ability of tumor cells to stimulate their own growth, replicate indefinitely, resist apoptosis, evade growth suppressors, and invade local tissue. (See Figure 1.)

At the tissue scale, we consider a heterogeneous microenvironment consisting of normal and tumor cells, extracellular matrix, and a pre-existing network of blood vessels. Oxygen released by the blood vessels diffuses in the microenvironment, providing the only source of nutrients to maintain cellular functions. Although not shown in Figure 1, tumor cells are further differentiated into a variety of phenotypes including quiescent, proliferative, migrating, hypoxic, apoptotic, and necrotic. In particular, proliferative and migratory phenotypes are orchestrated by complex signaling networks that integrate extracellular stimuli to produce regulatory responses^{20,25}. Here we consider the epidermal growth factor receptor (EGFR) pathway which is known to be upregulated in about 30% of all cancers³⁶. EGF is a protein that regulates a variety of cell functions, including cellular proliferation, differentiation, and survival. We assume that EGF is released by a quiescent tumor cell through autocrine signaling, so that it binds to surface receptors (EGF receptors - EGFR) on that same cell, triggering a cascade of protein interactions within the cell to produce a regulatory response that may give a cell proliferation or migration advantage. EGF also diffuses to nearby tumor cells. In this way, the macroscopic structure of tumor progression depends on the collective behavior of individual cells, each responding to external stimuli through sub-cellular events, governed by the supply of local oxygen and EGF availability.

Another important phenomenon considered in this model is the effect of the compressive stress accumulation within the growing tumor. These stresses play an important role on tumor evolution, regulating a variety of pathologies such as the collapse of blood and lymphatic vessels^{37,40}. Higher compressive stresses are known to inhibit cell mitosis and stimulate metastasis^{23,40}. Here we model proliferation inhibition induced by tumor growth. Thus, our hybrid model aims to integrate mechanisms from sub-cell (*nm*) to tissue (*mm*, *cm*) scales.

The three-scale avascular hybrid model developed here is depicted schematically in Figure 2. The ABM that describes the dynamics and movements of the normal and tumor cells is the core of the model. For simplicity, normal cells are kept in a state of homeostasis while cancer cells are differentiated into quiescent, proliferative, hypoxic, necrotic, apoptotic, and migrating phenotypes. Cell proliferation and migration are controlled by an intracellular signaling pathway in response to the availability of oxygen and EGF as well as by compressive stresses accumulated inside the tumor. The EGFR signaling pathway is modeled by a system of ordinary differential equations (ODEs) describing the stoichiometry of reactant and product protein complexes. The change in concentration of intracellular signal transducers triggers a cell proliferation or migration advantage. The biochemical cascade begins by activating the EGFR. This mechanism is modeled by a parabolic diffusion equation. The nutrient concentration also plays a key role on cell dynamics. In the present model, oxygen is considered the single source of nutrients, which is transported by diffusion from the original vascular network. Since oxygen transport is much faster than the cell cycle, it is modeled by a steady-state diffusion-reaction equation. We describe each of these models, as well as how they are coupled, in the following sections.

2.1. The Cell/Agent Based Model

The cellular scale is described through a lattice-free ABM, which allows for the tracking of each cell individually and describes the cell behavior in the microenvironment. The basic structure of the model has been inspired by that developed in Ref. 29 for the ductal carcinoma *in situ*. However, unlike Ref. 29, we consider both healthy (normal) and cancer cells which give rise to important mechanical interactions such as growth-induced stresses.

At time t , and position $\mathbf{x}_i$ lies the i^{th} cell, which is a physical object (an agent) that may interact with other cells and with the microenvironment through a variety of mechanical and chemical-driven forces (adhesion, repulsion, chemical attraction, etc.) which ultimately define the cell's lattice-free movement. Like many agent models^{12,13,29}, the cell geometry is quite simple: the i^{th} cell has a circular shape of radius R^i with a quasi-incompressible nucleus of radius R_N^i , as depicted in Figure 3. Cells are allowed to overlap their cytoplasms, mimicking a possible cell deformation due to interactions with other cells and with the microenvironment. Here, cell-cell adhesion and cell-cell repulsion are short-range interactions specified by an action radius R_A^i . Cell movement also depends on forces resulting from the interstitial fluid drag resistance and the haptotaxis towards extracellular matrix degradation. The effects of the compressive stresses that accumulate inside the tumor due to growth are also modeled, providing a feedback control mechanism on mitosis.

2.1.1. Cell Movement—The movement of each cell is driven by the force balance among cells, which depends on the cell position, time, and the microenvironment condition. The dynamic composition and structure of the extracellular matrix (ECM) and a variety of cell adhesion molecules mediate cell binding and migration. For simplicity, we do not consider ECM degradation and regeneration, ECM receptors and cell adhesion molecules, among other components that ultimately regulate cell-substrate adhesion. We simply assume that ECM composition and organization is heterogeneous, which yields haptotaxis. Since EGF is the only extracellular growth factor considered in the model, there is no cell movement due to chemotaxis (though it may be easily integrated in the present modeling framework). We consider that cell movement is determined by the action of the following four mechanisms:

1. cell-cell adhesive and cell-cell repulsive forces (denoted by $\mathbf{F}_{cca}$ and $\mathbf{F}_{ccr}$ respectively),
2. compression and resistance to compression forces (denoted by $\mathbf{F}_{ct}$ and $\mathbf{F}_{rtc}$ respectively) due to growth-induced stress,
3. force driven by haptotaxis ($\mathbf{F}_{hap}$),
4. drag force of interstitial fluid flow ($\mathbf{F}_d$).

Disregarding gravity, in a low speed interstitial flow the linear drag is given by $\mathbf{F}_d = -\eta \mathbf{v}$, where $\mathbf{v}$ is the cell velocity, and the constant η depends on the fluid viscosity. The forces $\mathbf{F}_{cca}$ and $\mathbf{F}_{ccr}$ depend on cell position and are defined through two interaction potentials (ϕ for adhesion, and ψ for repulsion^{6,12,27,29,35}). The adhesive and repulsive forces between the i^{th} and j^{th} cells are, respectively, given by

$$\mathbf{F}_{cca}^{ij} = -c_{cca} \nabla \varphi(\mathbf{d}^{ij}; R_A^i + R_A^j); \quad (2.1)$$

$$\mathbf{F}_{ccr}^{ij} = -c_{ccr} \nabla \psi(\mathbf{d}^{ij}; R_N^i + R_N^j, R^i + R^j), \quad (2.2)$$

in which $\mathbf{d}^{ij} = \mathbf{x}_j - \mathbf{x}_i$ is the distance between the i^{th} and j^{th} cells. The gradients of the potentials $\varphi(\mathbf{d}; R_A)$ and $\psi(\mathbf{d}; R_N, R)$ are simplified versions of those defined in Ref. 29, given by

$$\nabla \varphi = \begin{cases} \left(\frac{|\mathbf{d}|}{R_A} - 1 \right)^2 \frac{\mathbf{d}}{|\mathbf{d}|}, & 0 \leq |\mathbf{d}| \leq R_A; \\ \mathbf{0}, & \text{otherwise;} \end{cases} \quad \nabla \psi = \begin{cases} -\left(\frac{R_N |\mathbf{d}|}{R^2} - \frac{2|\mathbf{d}|}{R} + 1 \right) \frac{\mathbf{d}}{|\mathbf{d}|}, & 0 \leq |\mathbf{d}| \leq R_N; \\ -\left(\frac{|\mathbf{d}|^2}{R^2} - \frac{2|\mathbf{d}|}{R} + 1 \right) \frac{\mathbf{d}}{|\mathbf{d}|}, & R_N \leq |\mathbf{d}| \leq R; \\ \mathbf{0}, & \text{otherwise.} \end{cases}$$

To illustrate, Figure 5 shows $\nabla \varphi$ and $\nabla \psi$ with respect to the distance from the center of the cell for $R_N = 5.295 \mu\text{m}$, $R = 9.953 \mu\text{m}$ and $R_A = 12.083 \mu\text{m}$. Both have compact support defined by R_A and R , respectively. The adhesion between cells increases with their proximity and highest repulsive interaction takes place when $|\mathbf{d}| < R_N$, due to the small compressibility of cell nuclei. To avoid cell nuclei overlapping, the balance between $\nabla \varphi$ and $\nabla \psi$ must occur in the cytoplasm so that they are scaled by parameters c_{cca} and c_{ccr} . We assume that $c_{cca} = \gamma c_{ccr}$, where γ is the coefficient of proportionality.

We define the forces $\mathbf{F}_{ct}$ and $\mathbf{F}_{rct}$ to model the effects of the compressive stress accumulation within the growing tumor. Higher compressive stresses are known to inhibit cell mitosis and stimulate invasion^{23,40}. *In vitro* experiments conducted in Ref. 8 correlate stress accumulation with proliferation inhibition and an increase of apoptosis. We assume that the number of cells that leave the computational domain, denoted by $N_{out}(t)$, directly correlates with the growth-induced stress. In this way, $N_{out}(t)$ is used to regulate cell proliferation, as shown in the next section. Here, we define

$$\mathbf{F}_{ct}^i = -c_{ct} K(V, t) \nabla \varphi(\mathbf{d}_n^i; R_A^i); \quad (2.3)$$

$$\mathbf{F}_{rct}^i = -c_{rct} K(V, t) \nabla \psi(\mathbf{d}_n^i; R_N^i, R^i), \quad (2.4)$$

which are measures of stress accumulation within the tumor. In definitions (2.3) and (2.4), $\mathbf{d}_n^i$ is the normal distance between the center of the cell and the domain boundary, and the real-valued function $K(V, t) \in [0, 1]$ depends on the tumor volume at time t . We highlight two limiting cases:

- $K=0$: there is no stress accumulation and the tumor evolution does not depend on the medium deformation,
- $K=1$: the boundary behaves like a non-permeable incompressible membrane.

In this work, we set $K=0.1$, $c_{ccr}=c_{ct}$, $c_{rct}=10c_{cca}=10\gamma c_{ccr}$ for simplicity. (A detailed calibration procedure is the focus of a forthcoming effort.)

Migrating cells undergo haptotaxis towards the degradation of the ECM density, denoted by f . Given a heterogeneous field for f and the adhesion coefficient χ between the i^{th} migrating tumor cell and the ECM, the haptotactic force is defined as

$$\mathbf{F}_{hap}^i = -\chi \nabla f. \quad (2.5)$$

The equation of motion of each cell is obtained by summing the contribution of forces from all other cells, disregarding the inertial effects since the equilibrium is reached in a time frame much smaller than the cell cycle. Thus, with no chemical attractant and interaction with the ECM, and denoting by $N(t)$ the total number of cells, Newton's second law applied to the i^{th} cell yields

$$\mathbf{0} \approx m_i \dot{\mathbf{v}}_i = \sum_{\substack{j=1 \\ j \neq i}}^{N(t)} (\mathbf{F}_{cca}^{ij} + \mathbf{F}_{ccr}^{ij}) + \mathbf{F}_{ct}^i + \mathbf{F}_{rct}^i + \mathbf{F}_d^i + \mathbf{F}_{hap}^i. \quad (2.6)$$

The term under summation evaluates the cell-cell interactions while the last three terms represent the cell-microenvironment interactions. As in Refs. 13,29,35, given the drag force of interstitial fluid flow $\mathbf{F}_d^i = -\eta \mathbf{v}_i$, we obtain

$$\begin{aligned} \mathbf{v}_i &= \frac{1}{\eta} \left(\sum_{\substack{j=1 \\ j \neq i}}^{N(t)} (\mathbf{F}_{cca}^{ij} + \mathbf{F}_{ccr}^{ij}) + \mathbf{F}_{ct}^i + \mathbf{F}_{rct}^i \right) \\ &= \frac{-c_{ccr}}{\eta} \sum_{\substack{j=1 \\ j \neq i}}^{N(t)} (\gamma \nabla \varphi(\mathbf{d}^{ij}; R_A^i + R_A^j) + \nabla \psi(\mathbf{d}^{ij}; R_N^i + R_N^j, R^i + R^j)) \\ &\quad - \frac{c_{ccr}}{\eta} (10\gamma \nabla \varphi(\mathbf{d}^i; R_A^i) + \nabla \psi(\mathbf{d}^i; R_N^i, R^i)) + \frac{\chi}{\eta} \nabla f \end{aligned} \quad (2.7)$$

In Appendix A we present the overall steps to evaluate the force balance, the cellular velocities and their final positions.

2.1.2. Phenotypic heterogeneity—Every normal or tumor cell is an agent that may interact with the microenvironment and with other cells. We consider that normal cells are in homeostasis. As in Ref. 29, phenotypic heterogeneity in cancer cells is here characterized by the five states illustrated in Figure 6. These cell states result from an intricate interplay of nutrient availability, stimuli from the microenvironment, and interactions between the cells themselves. We also integrate negative proliferation feedback due to the accumulation of growth induced compressive stress, and we consider the intracellular decision process that gives a cell proliferation or migration advantage.

Cell-state changes from quiescent and proliferative states to hypoxic state occur only in response to a decrease in the availability in oxygen below the threshold, σ_H . For the present avascular model, hypoxic cells eventually die, entering the necrotic state at a rate $\beta_H \rightarrow \infty$. The transitions from the quiescent state to proliferative, migratory and apoptotic phenotypes are stochastic and driven by Poisson processes²⁹. By allowing interconversions to transition between any two phenotypic states, it was shown in¹⁹ that stochastic state changes promote an equilibrium of phenotypic proportions of cancer cells observed *in vivo*.

For small time intervals $[t, t + \Delta t]$, a cell in a quiescent state $\mathcal{Q}$ has the following probability to change to the state $\mathcal{P}$, $\mathcal{M}$ or $\mathcal{A}$:

$$P(\mathcal{P}|\mathcal{Q}) = 1 - \exp(-\alpha_P \Delta t), \quad \text{where } \alpha_P(t) = \bar{\alpha}_P \left(\frac{\sigma - \sigma_H}{1 - \sigma_H} \right) \left(1 - \frac{N_{\text{out}}^t}{N_{\text{out}}^{\text{max}}} \right), \quad (2.8)$$

$$P(\mathcal{M}|\mathcal{Q}) = 1 - \exp(-\alpha_M \Delta t), \quad \text{where } \alpha_M(t) = \bar{\alpha}_M \left(\frac{\sigma - \sigma_H}{1 - \sigma_H} \right), \quad (2.9)$$

$$P(\mathcal{A}|\mathcal{Q}) = 1 - \exp(-\alpha_A \Delta t), \quad \text{where } \alpha_A = \text{constant}. \quad (2.10)$$

The availability of oxygen in the microenvironment regulates cell migration and proliferation up to the threshold σ_H below which the cell does not have enough oxygen to neither undergo mitosis nor migrate. The third term in (2.8) models the influence of compressive stress accumulation on cell proliferation^{8,40}. Recall that we assume that the accumulation of growth-induced stresses within the tumor positively correlates with the number of cells that leave the computational domain, $N_{\text{out}}(t)$, provided $K(V, t) \geq 1$. Thus, as $N_{\text{out}}(t)$ grows, the compressive stress increases yielding a negative feedback on the cell proliferation. As $N_{\text{out}}(t)$ increases towards a certain threshold ($N_{\text{out}}^{\text{max}}$), cell proliferation is increasingly inhibited until eventually preventing cell mitosis. Proliferation and migration advantage is controlled by the cell response to the intracellular biochemical cascade (i.e., the EGFR signaling pathway) through the parameters $\bar{\alpha}_P$ and $\bar{\alpha}_M$, respectively. These parameters are usually null, except when the cell acquires proliferative or migratory advantage, when they become equal to one. Since we focus on the role of the intra-cellular

pathway on cell proliferation and migration, we assume for simplicity that α_A is constant. The rules that define the proliferative, necrosis, and apoptosis transitions are given in Figure 7. Once a tumor cell acquires the migratory phenotype, it is kept activated during a time τ_M after which the cell returns to the quiescent state.

2.2. The Intracellular Model

We focus on developing a multiscale framework that integrates a branch of the EGFR signaling pathway. EGF is a mitogen that regulates a variety of cell functions, having a distinguished role on cell proliferation and migration. We consider that EGF is secreted by quiescent cells and diffuses into the microenvironment where it reaches neighboring cells. We also assume that each cell is endowed with the intracellular model presented in Ref. 42, schematically illustrated in Figure 8. The pathway activation begins with the EGF binding to EGF receptors (EGFR), triggering a cascade of interactions involving 20 different molecules, leading to a regulatory response.

The signaling pathway model is mathematically described by a system of 20 coupled ordinary differential equations. The change in concentration of the i^{th} molecule, X_i , $i = 1, \dots, 20$, results from the mass-balance between all reactions producing and consuming X_i ⁴². For example, the reactions of the binding of EGF (X_1) to the cell surface receptor EGFR (X_2) that produces the complex EGF-EGFR (X_3), and the EGF-EGFR dimerization to (EGF-EGFR)₂ are respectively given by

in which k_i and k_{-i} are the forward and backward rate constants for reaction i defined as

$$v_1 = k_1 X_1 X_2 - k_{-1} X_3 \quad \text{and} \quad v_2 = k_2 X_3 X_3 - k_{-2} X_4.$$

Assuming that these reactions are the only causes of the change in the concentration of X_3 , we obtain

$$\frac{dX_3}{dt} = v_1 - 2v_2 = k_1 X_1 X_2 - k_{-1} X_3 - 2(k_2 X_3 X_3 - k_{-2} X_4) = (+1)(k_1 X_1 X_2 + 2k_{-2} X_4) + (-1)(k_{-1} X_3 + 2k_2 X_3 X_3).$$

Likewise, the balance equations describing the production and consumption of the EGFR pathway molecules are described by the system of ODEs presented in Ref. 42. Two key molecules, ERK (extracellular signal-regulated kinase) and PLC $_{\gamma}$ (phospholipase C-gamma), play crucial roles on regulating proliferation and migration. Migration and proliferation cannot occur simultaneously¹⁶. Indeed, it has recently shown that proliferative signaling and activation of migration are organized into two different meta-pathways, requiring distinct cell investment strategies³⁰. Given the thresholds T_{PLC} and T_{ERK} , the signaling pathway control process may give a cell an advantage to proliferate or to migrate, according to the rule defined in Table 1. Otherwise, both $\bar{\alpha}_P$ and $\bar{\alpha}_M$ are null.

The choice of the decision thresholds, T_{PLC} and T_{ERK} , must be calibrated to represent the desired tumor expansion pattern. For a given T_{PLC} , decreasing T_{ERK} would lead to a faster growth. However, this behavior may not be smooth, as indicated in Ref. 4, and may depend on other extracellular cues. This is an issue that deserves further investigation.

2.3. Macroscopic models: oxygen and EGF transport

At the macroscopic scale, the constituents of heterogeneous tissues are usually described by a set of scalar fields representing the volume fractions of each constituent. Their evolution is governed by mass balance equations and equations describing mechanical deformation and stress. In the present formulation, only the evolutions of oxygen (σ), and EGF concentrations at the tissue scale are modeled.

To define the tissue-scale model, let Ω denote an open bounded, Lipschitz domain in $\mathbb{R}^d$ with smooth boundary $\partial\Omega$ in which living tissue evolves due to the supply of oxygen from the original vascular system. Let the time t be defined in the interval $(0, T_{tissue}]$, in which T_{tissue} is the maximum evolution time at the tissue scale. Oxygen dispersion in the tumor microenvironment is much faster than tumor growth dynamics^{5,17} so it is considered a quasi-stationary process. The oxygen concentration at a point $\mathbf{x} \in \Omega$ at a time $t \in (0, T_{tissue}]$ is governed by a mass conservation condition of the form,

$$\nabla_{\mathbf{x}} \cdot D \nabla_{\mathbf{x}} \sigma(\mathbf{x}, t) - \Lambda(\mathbf{x}, t) \sigma(\mathbf{x}, t) = 0, \quad (2.11)$$

in which $\nabla_{\mathbf{x}}$ is the tissue-level gradient operator, D is the oxygen diffusion coefficient and $\Lambda(\mathbf{x}, t) > 0$ is the oxygen uptake rate, which depends on averages of sub-scale events. Tumor and healthy cells have different oxygen uptake rates, denoted by λ and λ_b , respectively. General boundary conditions are

$$\sigma(\mathbf{x}, t) = \sigma_0(\mathbf{x}, t), \mathbf{x} \in \partial\Omega_1, \quad \mathbf{n} \cdot \nabla_{\mathbf{x}} \sigma(\mathbf{x}, t) = 0, \mathbf{x} \in \partial\Omega_2, t \geq 0, \quad (2.12)$$

with $\bigcup_{i=1}^2 \partial\Omega_i \equiv \partial\Omega$, and $\mathbf{n}$ an exterior unit normal vector to Ω_2 . With these definitions, we assume that an original blood vessel lines the boundary Ω_1 and there is no oxygen flux on Ω_2 .

We assume that EGF is secreted by quiescent cells and diffuses in the microenvironment according to

$$\frac{\partial[\text{EGF}]}{\partial t} = \nabla_{\mathbf{x}} \cdot D_{\text{EGF}} \nabla_{\mathbf{x}} [\text{EGF}] + F_{\text{EGF}}, \quad (2.13)$$

in which D_{EGF} is the EGF diffusion coefficient and F_{EGF} is the autocrine EGF source term. The EGF diffusion coefficient is very small, much smaller than the oxygen diffusion coefficient ($D_{\text{EGF}} \ll D$), so that the EGF stimulus reaches only neighboring cells,

representing the cell-cell and cell-extracellular matrix (juxtacrine/paracrine) communication. We assume that the concentration of EGF in the boundary is zero.

Both Λ and F_{EGF} link the tissue and cell scales. We transfer information between these two scales with the help of two nested square lattices. Let the coarser grid $\mathcal{M}_H$, with characteristic length H , be used to solve the tissue scale models (2.11) and (2.13). We define an auxiliary and more refined grid $\mathcal{M}_h$, with characteristic length h such that $h = H/N$, in which N is an integer and even number. In all experiments performed here, we use $N = 10$. At each node p of $\mathcal{M}_H$ that lies at $\mathbf{x}_p = (x_p, y_p)$, we define a Moore neighborhood of range $N/2$, denoted by $\mathbb{M}_p^{N/2}$. Thus, $\mathbb{M}_p^{N/2}$ defines the square-shaped region of $\mathcal{M}_h$ containing the grid points $\mathbf{x} \in \{(x, y) : |x - x_p| < Nh/2, |y - y_p| < Nh/2\}$. We evaluate $\Lambda(\mathbf{x}_p, t)$ and $F_{EGF}(\mathbf{x}_p, t)$ by upscaling cell data of all cells belonging to $\mathbb{M}_p^{N/2}$ through averaging the cell properties. To translate information from the tissue scale to cell scale, we first identify the location of each cell with respect to the partition of $\mathcal{M}_H$. Then, given the four nodes of $\mathcal{M}_H$ surrounding each cell, we linearly interpolate the oxygen and EGF concentrations at these nodes to the cell position.

3. Simulations

We investigate the behavior of the multiscale model, and, in particular, the effects of EGF dispersion and intracellular control on tumor growth. The solution process of the hybrid model is schematically shown in Figure 9. After initializing the ABM, the oxygen and EGF concentrations are updated and translated to each tumor cell afterwards. The signaling pathway model is solved for each cell in accordance with external cues. The cell model performs phenotypic transitions, if needed, and updates the ongoing apoptosis, necrosis, migration, and proliferation processes. The balance of forces is carried out for every cell (which is the more computationally expensive part of the model), yielding updated cell velocities and positions. Then, new representative values of oxygen uptake rate and EGF production are translated back to each macroscopic grid point, yielding a new configuration of oxygen and EGF dispersion. The time evolution continues until the end of the predetermined length of time is reached. All these modules are implemented in C_{++} . The partial differential equations are solved using a finite difference model (central differences) and the signaling pathway model is solved using a fourth-order Runge-Kutta method.

In all numerical experiments, we consider normal cells randomly placed in a circular tissue substrate of radius equal to $250 \mu m$, with two proliferative and two quiescent cells at the center. The computational domain for the (continuum) macroscale tissue environment is quadrangular with side $500 \mu m$. We assume that there is a basal vasculature at the boundary providing a constant source of oxygen ($\Omega \equiv \Omega_1$). Since EGF is assumed to be released by quiescent cells, its concentration vanishes on the boundary where there are only healthy cells. We use the notation depicted in Figure 10 to display cell differentiation obtained with the ABM. Model parameters are defined in Tables 2–4. We use the ABM parameters defined in Ref. 29. For the signal transduction pathway, the parameters were extracted from Ref. 42. In all the following experiments we solve the partial differential equations on a regular grid with 50 divisions in each direction ($H = 10 \mu m$), and time step $\Delta t = 1 h$.

To investigate the effects of the intracellular phenotypic and proliferation inhibition (due to the accumulation of compressive stresses) on the spatiotemporal development of the tumor, we designed three numerical experiments:

Experiment 1: Cells may acquire a proliferation advantage and there is no proliferation inhibition due to accumulation of compressive stresses inside the tumor. Mathematically, these assumptions affect the ability to change its state in the following manner:

$$\alpha_P(t) = \bar{\alpha}_P \left(\frac{\sigma - \sigma H}{1 - \sigma H} \right), \alpha_M(t) = 0, \text{ and } F_{\text{hap}} = 0;$$

Experiment 2: Cells may acquire a proliferation advantage, which is inhibited due to accumulation of compressive stresses inside the tumor. These assumptions amount to set

$$\alpha_P(t) = \bar{\alpha}_P \left(\frac{\sigma - \sigma H}{1 - \sigma H} \right) \left(1 - \frac{N_{\text{out}}^t}{N_{\text{out}}^{\text{max}}} \right), \alpha_M(t) = 0, \text{ and } F_{\text{hap}} = 0;$$

Experiment 3: Cells may acquire a proliferation or migration advantage, and proliferation is inhibited due to accumulation of compressive stresses inside the tumor, yielding

$$\alpha_P(t) = \bar{\alpha}_P \left(\frac{\sigma - \sigma H}{1 - \sigma H} \right) \left(1 - \frac{N_{\text{out}}^t}{N_{\text{out}}^{\text{max}}} \right), \text{ and } \alpha_M(t) = \bar{\alpha}_M \left(\frac{\sigma - \sigma H}{1 - \sigma H} \right).$$

In all experiments, we perform 100 realizations using the initial conditions shown in Figure 11, which represent a small tumor in the center of the domain. As experiment 3 requires the definition of the ECM density, we assume that $f \in \mathcal{U}[0, 5; 1]$ is the non-dimensional ECM density.

3.1. Experiment 1

Figure 12 shows one realization of the tumor evolution without feedback from the microenvironment stresses. Beginning with a small tumor at the center of the tissue substrate and a uniform oxygen distribution, the tumor develops and gradually uptakes the available oxygen. At $t = 4.17$ days, the tumor is mainly composed of proliferative cells, which represents the early stages of an avascular tumor. The high growth rates yield a rapid decrease of the oxygen concentration towards the tumor center, where the oxygen level is not sufficient to maintain cell viability. At $t = 8.33$ days, a necrotic core begins to develop so that a three layer phenotype pattern is formed: an internal region representing the necrotic core, surrounded by quiescent cells, and a proliferative external rim¹⁸. Because the EGF released by quiescent cells is transported by a small diffusion coefficient, it only reaches neighboring cells. At $t = 16.67$ days, tumor cells occupy the whole domain, and a calcified necrotic core is visible. At $t = 33.34$ days, the red core indicates that the calcification process has completed.

3.2. Experiment 2

Figure 13 shows one realization of the tumor evolution with feedback from the microenvironment stresses. The negative feedback has a strong impact on tumor development. In particular, by comparing Figures 12 and 13 it is readily apparent that the growth is much slower and more heterogeneous as areas that experience an accumulation of stress have a reduction in proliferation. This negative feedback ultimately disrupts the rounded tumor growth pattern, so that at 8.33 days the tumor displays signs of invasion, which can be viewed as a strategy to increase oxygen contact area. However, as the tumor grows, a more intense negative feedback on the proliferation may eventually prevent growth, which has been observed experimentally^{37,40}. At $t = 33.34$ days, there are just a few proliferative cells in the domain and the tumor morphology still shows some branching.

Figure 14a shows the evolution of the mean of all tumor phenotypes after 100 model realizations. In general, as the tumor cells multiply, they take the place of the normal cells. The number of proliferative cells reaches a plateau and decreases at the end of the simulation. With the decrease of oxygen concentration, the necrotic core appears around 350 h and grows slowly, eventually overcoming the number of proliferative cells in less than 200 h. The evolution of individual phenotypes are shown in Figure 14b–f. The evolution of necrotic, quiescent and normal cells show quite regular standard deviations. The stochastic transitions to proliferative and apoptotic states give rise to a high variability of the evolution, which steadily grows in time. The decrease of the number of proliferative cells at the end of the simulation is more visible in Figure 14d, showing a sharp tendency towards growth arrest, typical of avascular tumors.

In Figure 15 we compare the evolution of the mean of the cellular phenotypes obtained by fixing $\bar{\alpha}_P$ at four different values (0.25; 0.52; 0.75; 1.0), i.e., without considering any proliferation advantage/disadvantage provided by the intracellular model. Higher $\bar{\alpha}_P$ yields a higher maximum mean value of proliferative cells, as expected. In all cases, the number of proliferative cells eventually vanishes, characterizing the growth arrest. The higher peak of proliferative cells obtained with $\bar{\alpha}_P = 1$ yields a faster growth arrest and a slightly larger necrotic core. Overall, the evolution pattern obtained without the intracellular control is qualitatively similar, with a stable necrotic core and no proliferative cells (growth arrest) after 500h. In particular, Figure 16 shows a detailed evolution of necrotic, quiescent, proliferative and apoptotic cells for $\bar{\alpha}_P = 0.52$ using 100 realizations, together with the corresponding standard deviations. By comparing with Figure 14, it is remarkable that the proliferation advantage introduced by the signaling pathway also yields a higher variance of the number of proliferative cells. This ultimately leads to a more irregular and heterogeneous tumor evolution branching pattern, keeping the three layer structure typical of avascular tumors, as displayed in Figure 13.

3.3. Experiment 3

Figure 17 shows one realization of the tumor evolution at $t = 4.17$, 8.33, and 12.5 days, in which tumor cells may acquire a proliferation or migration advantage, and are subject to the proliferation negative feedback from the microenvironment stresses. As the tumor grows, it gradually consumes the oxygen available in the microenvironment, and EGF dispersion is

restricted to the vicinity of the cells that release the growth factor. At $t = 8.33$ days, a two layer pattern emerges: an inner region of quiescent cells and an outer layer where most cells are proliferative (green) and migratory (yellow). The migration process favors a high mobility tumor expansion. At $t = 12.5$ days the oxygen concentration towards the center of the domain is not enough to sustain cell viability so that the necrotic core emerges. Eventually, necrotic cells undergo calcification. The simulation is halted at this time since the tumor mass now occupies the whole domain. Comparing with Experiment 2, tumor growth is much faster, its growth pattern is more rounded, and the tumor mass is also interleaved with normal cells. Overall, the migration process has a substantial impact on tumor invasiveness and shape.

Figure 18 shows the evolution of the mean of all individual tumor phenotypes and of the migrating cells after 100 model realizations. Comparing Figures 14a and 18a, we notice that the absence of the migration process yields a longer tumor growth. Even in small quantities, the number of migrating cells grows steadily, speeding up the tumor growth which eventually halts due to the lack of additional oxygen source.

4. Conclusions

We have developed a hybrid three-scale avascular tumor growth model which integrates phenomena occurring at the tissue, cell and intracellular scales. We model each spatial scale with different mathematical/computational formalism yielding a modular framework in which each module can be easily exchanged or expanded. With this approach we are able to track how cells process microenvironment stimuli and produce regulatory responses, which eventually give rise to a more heterogeneous tumor expansion.

At the cellular level, the agent-based model describes cell mobility, mitosis, apoptosis, necrosis, cell-cell adhesion and repulsion, cell compression due to growth, and cell contact with surrounding microenvironment. At the tissue scale, oxygen and epidermal growth factor concentration are modeled through reaction-diffusion equations. Those microenvironment stimuli are translated to cell scale, driving a variety of phenomena that ultimately regulate cell phenotypes and tumor expansion. A specific sub-cell model is introduced to regulate cell proliferation or migration advantage. The EGFR signaling pathway describes a cascade of biochemical reactions, which is modeled by a system of ordinary equations. Solution of these equations defines the decision process that confers (or does not) the proliferation or migration advantage to the cell. Thus, the final regulation of the proliferative phenotype depends on the intracellular driven proliferation advantage, and the inhibition due to both nutritional stress and cell compression. Likewise, the migrating phenotype results from the integration of the intracellular driven migration advantage and the nutritional stress.

Simulations have shown that the inhibition of proliferation due to the accumulation of growth-induced compressive stresses within the tumor may give rise to a branching tumor growth morphology, with fingers appearing at the earliest stages of the tumor expansion. Likewise, the integration of the EGFR signaling pathway to drive cell proliferation or migration advantages provides a substantial change on the overall phenotypic heterogeneity

of the tumor. In this way, the developed hybrid framework that integrates intracellular, cellular, and tissue scales is able to produce a rich variety of tumor behaviors.

We believe that our multiscale model is a start and fundamental platform that will allow to investigate a variety of cancer mechanisms occurring at molecular, cell or tissue level as well as potential target specific therapies and their effects through other pathways. There are many avenues of investigation to this end. Among them we highlight the calibration and validation of the present multiscale avascular model by integrating *in vivo* and *in vitro* experiments for a selected type of cancer. To test different *in silico* scenarios, the present model could be extended to integrate angiogenesis, extracellular matrix heterogeneity, stem cells, explicit mechanical deformation, metabolic networks and other signaling pathways, among other features recognized to play important roles in tumor expansion.

There is a clear need for reliable quantitative methods that can predict and assess the response of tumors to therapy^{44,45}, and to do so with great sensitivity and specificity, earlier in clinical follow-up; such an approach would have profound implications for diagnosis, therapy, and prognosis. We believe that the present effort is a step towards enhancing computational and mathematical model framework with the potential to unfold complex multiscale phenomena in cancer.

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Appendix A

ABM algorithms

Algorithm 1

Cell Apoptosis

Input: i, A_0^i, τ_A

Result: Update of cellular apoptosis

if $t > \tau_A/2$ **then**

$A^i(t) \leftarrow A^i(t) - \frac{A_0^i}{\tau_A}$ # decreasing cell area;

end if

if $t = \tau_A$ **then**

Cell is removed from the simulation;

end if

Algorithm 2

Force balance, cellular velocity and position

Result Update cell positions**while** $t \leq 60 \text{ min}$ **do** **for** $i = 1$ **to** $N(t)$ **do** **for** $j = 1$ **to** $N(t)$ **do** **if** $i = j$ **then**

|

continue; **end if**

$$F_{cca}^{ij} \leftarrow -c_{cca} \nabla \varphi(\mathbf{x}_j - \mathbf{x}_i; R_A^i + R_A^j)$$

$$F_{ccr}^{ij} \leftarrow -c_{ccr} \nabla \psi(\mathbf{x}_j - \mathbf{x}_i; R_N^i + R_N^j, R_i + R_j)$$

$$\mathbf{F}^i \leftarrow \mathbf{F}^i + (F_{cca}^{ij} + F_{ccr}^{ij})$$

end for

$$\mathbf{F}_{ct}^i \leftarrow K(V, t) \cdot [-c_{ct} \nabla \varphi(\mathbf{d}_n^i; R_A^i)]$$

$$\mathbf{F}_{rct}^i \leftarrow K(V, t) \cdot [-c_{rct} \nabla \psi(\mathbf{d}_n^i; R_N^i, R_i)]$$

$$\mathbf{F}_{hap}^i \leftarrow \chi \nabla f$$

$$\mathbf{F}^i \leftarrow \mathbf{F}^i + (\mathbf{F}_{cba}^i + \mathbf{F}_{cbr}^i + \mathbf{F}_{ct}^i + \mathbf{F}_{rct}^i + \mathbf{F}_{hap}^i)$$

end for **for** $i = 1$ **to** $N(t)$ **do** | $\mathbf{v}^i \leftarrow \mathbf{F}^i$; **end for**

$$\Delta t \leftarrow \frac{1}{\max \|\mathbf{v}^i\|};$$

for $i = 1$ **to** $N(t)$ **do** | $\mathbf{x}^i \leftarrow \mathbf{x}^i + \mathbf{v}^i \cdot \Delta t$; **end for** $t \leftarrow t + \Delta t$ **end while****Algorithm 3**

Cell Necrosis

Input i, A_0^i, A_N^i (cell nucleus area), τ_{NL}, τ_C

Result Update of cellular necrosis

if $t < \tau_{NL}$ **then**

$$A^i \leftarrow A_0^i \left(1 + \frac{t}{\tau_{NL}} \right) \text{ \# cell swelling;}$$

end if

if $t = \tau_{NL}$ **then**

$$A^i \leftarrow A_N^i \text{ \# cell lysing;}$$

end if

if $t > \tau_{NL}$ **and** $t > \tau_C$ **then**

$$C \leftarrow \frac{t}{\tau_C} \text{ \# cell calcification;}$$

end if

if $t > \tau_C$ **then**

$$C = 1;$$

end if

Algorithm 4

Cell Mitosis

Input: $i, A_0^i, \mathbf{x}_i, R_{N0}^i, \tau_P, \tau_{G1}$

Result: Update cellular mitosis

if $t = (\tau_P - \tau_{G1})$ **then**

$$A^i(t=0) \leftarrow \frac{1}{2} A_0^i \text{ \# daughter cells have half area of the parent cell;}$$

$$A^k(t=0) \leftarrow \frac{1}{2} A_0^i;$$

Sample a vector $\mathbf{V}$ centered on $\mathbf{x}_i$ and modulus $R_{N0}^i \frac{\sqrt{2}}{2}$

$$\mathbf{x}_i \leftarrow \mathbf{x}_i + \mathbf{V};$$

$$\mathbf{x}_k \leftarrow \mathbf{x}_i - \mathbf{V};$$

$$S^i \leftarrow G1 \text{ \# daughter cells enter G1 cell-cycle phase;}$$

$$S^k \leftarrow G1;$$

end if

if $S^i = G1$ **and** $t > \tau_P$ **then**

$$A^i(t) \leftarrow \frac{1}{2} A_0^i \left(1 + \frac{\tau_{G1} + (t - \tau_P)}{\tau_{G1}} \right);$$

end if

```

if  $S^i = G1$  e  $t = \tau_p$  then
|    $S^i \leftarrow \mathcal{Q}$  # cell enter G0 cell-cycle phase;

end if

```

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Fig. 1.

Schematic description of the key biological characteristics included in the model. The tumor microenvironment is highly heterogeneous, consisting of normal and cancer cells, extracellular matrix, and blood vessels. Original blood vessels provide oxygen that maintains cell viability. The box on the left presents an enlargement of an individual tumor cell indicating its interactions with the surrounding milieu via autocrine and paracrine signaling as well uptake of oxygen.

Fig. 2. Schematic description of the model. The nutrient and EGF dispersions that occur at the tissue scale are modeled by partial differential equations. At the cell scale, healthy and cancer cells are described using an agent based model. The intracellular phenomena that regulate cell proliferation and cell migration are described by a mass action model of the biochemical signaling pathway through a system of ordinary differential equations.

Fig. 3.

Geometric cell description of the i^{th} cell, in which R^i is the cell radius, R_N^i is the nuclear radius, and R_A^i is the action radius. The irregular dashed curve represents one possible cell geometry which is simplified by the assumption of the circular cell shape. This approach is inspired by Ref. 29.

Fig. 4.

Cell-cell adhesion and repulsion are represented by pairwise forces F_{cca}^{ij} and F_{ccr}^{ij} , respectively. Tumor growth gives rise to compression and resistance to compression forces (F_{ct}^i and F_{rct}^i , respectively), and all moving cells undergo a drag force of interstitial fluid flow (F_d). ECM heterogeneity may yield haptotaxis to migrating cells (F_{hap}). This approach is inspired by Ref. 29.

Fig. 5. Gradients of potential functions ϕ (a) and ψ (b) with respect to the distance from the center of the cell for $R_N = 5.295 \mu\text{m}$, $R = 9.953 \mu\text{m}$ and $R_A = 12.083 \mu\text{m}$. Both cancel out for distances greater than the action radius R_A .

- Q **quiescent cell**: cell in the phase $G0$ of the cell cycle
- P **proliferative cell**: cell undergoing mitosis
- H **hypoxic cell**: cell undergoing nutrient stress
- A **apoptotic cell**: cell undergoing a programmed cell death
- N **necrotic cell**: cell undergoing nutrient shortage
- M **migrating cell**: cell undergoing migration

Fig. 6.

Schematic illustration of cancer cell transitions, that can be deterministic (black arrows) or stochastic (red arrows). α_P , α_M and α_A are intensity functions of the stochastic processes. Cells become hypoxic if the nutrient concentration σ drops below the threshold σ_H . Hypoxic cells become necrotic automatically (at a rate $\beta_H \rightarrow \infty$) since we are considering an avascular model. Apoptotic cells are removed from the simulation after τ_A , time at which the processes of organized cell degradation and phagocytosis take place. Quiescent cells may achieve proliferation or migration advantage. Once a cell receives the proliferation signal, the cell cycle begins, lasting a time τ_P in which mitosis (M) takes place after the S and $G2$ phases. The daughter cells enter the quiescent state ($G0$) after the growth phase $G1$, that lasts a time τ_{G1} . Migration proceeds for a time τ_M after which cells return to the quiescent state.

(a) Proliferation rules. Cell spends $\tau = \tau_P - \tau_{G1}$ in cell cycle phases S , G_2 and M . After the time τ , two identical new cells are created, each one with half the area of the parent cell. They then spend time τ_{G1} in the G_1 phase in order to reach cell maturity and finally enter G_0 phase (or Q).

(c) Apoptosis rules. Programmed cell death involves two main processes. First, an organized cell degradation occurs (blebbing, nuclear fragmentation, etc.), followed by the phagocytosis of the resulting apoptotic bodies^{15,34}. We steadily decrease the cell diameter until it completely disappears. The whole process lasts a period τ_A , after which the cell is removed from the simulation.

(b) 2D cell division. One of the daughter cells is randomly positioned at a distance R_{N1} from the parent cell, where R_{N1} is the nuclear radius of a cell with half the area of the parent cell. The other daughter cell is placed in the opposite direction.

(d) Necrosis rules. Nutritional deprivation yields hypoxia which eventually leads to necrosis during avascular tumor evolution. First, the cell swells before lysing during a time τ_{NL} ³⁴. Afterwards, the cell membrane breaks, cell size decreases to the nucleus size and the calcification process takes place³⁹. Calcium concentration increases until time τ_C , indicated by the red cell.

Fig. 7.

Cell transition rules (inspired by Ref. 29) and 2D cell division. The corresponding algorithms are shown in Appendix A.

Fig. 8.

Schematic illustration of a simplified version of the EGFR signaling pathway, in which only the key molecules included in the model are displayed. The EGF binds to the cell surface EGFR, triggering a biochemical cascade involving key signaling molecules: enzyme PLC_{γ} and protein kinases PKC, Raf, MEK and ERK. The black, grey and dashed arrows indicate the signaling pathway, the cross-talk junctions with other pathways and the biological regulatory response, respectively.

Fig. 9.

Schematic representation of the model solution process. Given an initial configuration of the ABM, the microenvironment conditions are updated by solving the reaction-diffusion models. Oxygen and EGF concentrations are translated to the cell scale, and the intracellular model is solved to determine whether each cell has achieved a proliferation or migration advantage. Phenotypic transitions are carried out according to the ABM rules. Cell forces are updated and their balances lead to new cell positions. Cell attributes are translated back to the tissue scale, completing the time-step evolution.

Fig. 10.

Notation used in the simulations. Healthy cells are denoted by $\mathcal{N}ormal$, and tumor cells are differentiated by phenotype: quiescent ($\mathcal{Q}$), proliferative ($\mathcal{P}$), migrating ($\mathcal{M}$), apoptotic ($\mathcal{A}$), early stages of necrosis ($\mathcal{N}$) and three increasingly higher levels of calcification.

Fig. 11. Initial condition: (left) normal cells randomly placed, and four tumor cells at the center (two proliferative and two quiescent cells); (center) uniform oxygen field; (right) EGF released by the quiescent cells.

(a) Simulation at 4.17 days: the tumor is predominantly formed by proliferative cells.

(b) Simulation at 8.33 days: the tumor presents a three phenotype layer pattern, and a necrotic core is now visible.

(c) Simulation at 16.67 days: calcification of the necrotic core begins, and there is a substantial decrease of the oxygen concentration towards the tumor center.

(d) Simulation at 33.34 days: calcification of the necrotic core has completed.

Fig. 12.

Experiment 1: One realization of the ABM model (left column), oxygen dispersion (middle column), and EGF dispersion (right column). Cells are subject to pathway proliferation control and no negative feedback from compressive stress accumulation due to growth.

Fig. 13.

Experiment 2: One realization of the ABM model (left column), oxygen dispersion (middle column), and EGF dispersion (right column). Cells subject to the pathway proliferation control and negative feedback from compressive stress accumulation due to growth.

Fig. 14.

Evolution of the mean of individual cell phenotypes. $\mathcal{N}$, $\mathcal{Q}$, $\mathcal{P}$ and $\mathcal{H}$ stand for the number of cells in the necrotic, quiescent, proliferative and healthy phenotypes, respectively. The shaded areas represent the standard deviations at each evolution time.

Fig. 15.

Evolution of the mean of individual phenotypes without considering the signaling pathway, and setting $\bar{\alpha}_P$ to different values. Although the general behavior is quite similar, some differences in the growth dynamics are visible. Higher $\bar{\alpha}_P$ values lead to faster and greater growth of proliferative cells. As the availability of oxygen is limited, a greater number of proliferative cells cause a faster consumption of the oxygen supply, leading to faster stagnation of tumor growth.

(a) Evolution of necrotic cells.

(b) Evolution of quiescent cells.

(c) Evolution of proliferative cells.

(d) Evolution of apoptotic cells.

Fig. 16.

Evolution of the mean of individual cell phenotypes for $\bar{\alpha}_P = 0.52$. The shaded areas represent the standard deviations at each evolution time. These variabilities are remarkably smaller than those that emerge from the intracellular control (see Figure 14).

(a) Simulation at 4.17 days: the tumor is predominantly formed by proliferative cells.

(b) Simulation at 8.33 days: a two phenotype layer pattern emerges from the tumor growth, with quiescent cells in an inner region and an outer region with proliferative and migrating cells. .

(c) Simulation at 12.5 days: the rapid growth of the tumor leads to a substantial decrease in the availability of oxygen towards the center of the tumor, giving rise to the necrotic core, where the first signs of calcification appear.

Fig. 17.

Experiment 3: One realization of the ABM model (left column), oxygen dispersion (middle column), and EGF dispersion (right column). Cells are subject to the pathway control and negative proliferation feedback from compressive stress accumulation due to growth.

Fig. 18.

Evolution of individual cell phenotypes (a). The vertical axis display the number of cells in the normal, quiescent (Q), proliferative (P) and necrotic (N) phenotypes. The details of the evolution of the mean of the number of migrating cells and corresponding standard deviations at each evolution time (b). Although the number of migratory cells is small, the tumor dynamics are greatly accelerated.

Table 1

Downstream response induced by the EGFR signaling pathway; [ERK] and [PLC γ] indicate the concentrations of ERK and PLC γ , respectively.

Proliferation advantage ($\bar{\alpha}_P = 1$)	Migration advantage ($\bar{\alpha}_M = 1$)
$\frac{d[\text{PLC}\gamma]}{dt} < T_{\text{PLC}}$ and $\frac{d[\text{ERK}]}{dt} > T_{\text{ERK}}$	$\frac{d[\text{PLC}\gamma]}{dt} > T_{\text{PLC}}$ and $\frac{d[\text{ERK}]}{dt} < T_{\text{ERK}}$

Table 2

Model parameters.

Parameter	Meaning	Value	Ref.
R, R_N	cell radius and cell nuclear radius	$9.953 \text{ e } 5.295 \mu\text{m}$	²⁹
R_A	action radius	$1.214R$	²⁹
c_{ccr}	cell-cell repulsion coefficient	$5 \eta \mu\text{m}/\text{min}$	estimated
γ	cell-cell adhesion scale	0.0488836	²⁹
$M_{\text{out}}^{\text{max}}$	maximum number of cells that leave the domain	1500	estimated
σ_B	oxygen concentration at the boundary	0.6	estimated
σ_H	hypoxic threshold	0.2	²⁹
α_A	$\mathcal{Q} \rightarrow \mathcal{A}$ transition intensity	$0.0012728 \text{ hr}^{-1}$	²⁹
τ_P	cell cycle time	18 h	²⁹
τ_{G1}	G_1 cell cycle phase time	9 h	²⁹
τ_A	apoptosis time	8.6 h	²⁹
τ_{NL}	lysing time	6 h	²⁹
τ_C	necrosis time	360 h	²⁹
τ_M	maximum motility time	1 h	estimated
χ	haptotaxis coefficient	$25 \eta \mu\text{m}/\text{min}$	estimated
D	oxygen diffusion coefficient	$1000 \mu\text{m}^2/\text{h}$	²⁹
λ	oxygen uptake rate of tumor cells	0.1 min^{-1}	²⁹
λ_b	oxygen uptake rate of healthy cells	0.01λ	²⁹
D_{egf}	EGF diffusion coefficient	$1 \times 10^{-12} \mu\text{m}^2/\text{h}$	estimated
F_{egf}	EGF concentration secreted by quiescent cells	$2.65 \text{ nM}/\text{s}$	⁴²
T_{PLC}	threshold for proliferation advantage	$-1 \times 10^{-4} \text{ nM}/\text{s}$	estimated
T_{ERK}	threshold for migration advantage	$1.2 \times 10^{-5} \text{ nM}/\text{s}$	estimated

Table 3

Kinetic equations and initial conditions (extracted from⁴²). Reaction and parameter definitions are given in Table 4.

Reactant	Molecule	Initial condition	kinetic equation
X_1	EGF	tissue scale PDE	$d(X_1)/dt = -v_1$
X_2	EGFR	80	$d(X_2)/dt = -v_1$
X_3	EGF-EGFR	0	$d(X_3)/dt = v_1 - 2v_2$
X_4	(EGF-EGFR) ₂	0	$d(X_4)/dt = v_2 + v_4 - v_3$
X_5	EGF-EGFR-P	0	$d(X_5)/dt = v_3 + v_7 - v_4 - v_5$
X_6	PLC $_{\gamma}$	10	$d(X_6)/dt = v_8 - v_5$
X_7	EGF-EGFR-PLC $_{\gamma}$	0	$d(X_7)/dt = v_5 - v_6$
X_8	EGF-EGFR-PLC $_{\gamma}$ -P	0	$d(X_8)/dt = v_6 - v_7$
X_9	PLC $_{\gamma}$ -P	0	$d(X_9)/dt = v_7 - v_8 - v_9 - v_{10}$
X_{10}	PLC $_{\gamma}$ -P-I	0	$d(X_{10})/dt = v_9$
X_{11}	PKC	10	$d(X_{11})/dt = -v_{10}$
X_{12}	PKC*	0	$d(X_{12})/dt = v_{10} - v_{11}$
X_{13}	Raf	100	$d(X_{13})/dt = -v_{11}$
X_{14}	Raf*	0	$d(X_{14})/dt = v_{11} - v_{12} - v_{14}$
X_{15}	MEK	120	$d(X_{15})/dt = v_{13} - v_{12}$
X_{16}	MEK-P	0	$d(X_{16})/dt = v_{12} + v_{15} - v_{13} - v_{14}$
X_{17}	MEK-PP	0	$d(X_{17})/dt = v_{14} - v_{15} - v_{16} - v_{18}$
X_{18}	ERK	100	$d(X_{18})/dt = v_{17} - v_{16}$
X_{19}	ERK-P	0	$d(X_{19})/dt = v_{16} + v_{19} - v_{17} - v_{18}$
X_{20}	ERK-PP	0	$d(X_{20})/dt = v_{18} - v_{19}$

Table 4

Reaction and parameter definitions. Concentrations and the Michaelis-Menten constants (K) are given in $[nM]$. First- and second-order rates are given in $[s^{-1}]$ and $[nM^{-1}s^{-1}]$, respectively, and V are given in $[nMs^{-1}]$. (Extracted from⁴².)

Reaction	Equation	Kinetic parameters		Ref.
v_1	$k_1 X_1 X_2 - k_{-1} X_3$	$k_1 = 0.003$	$k_{-1} = 0.06$	24
v_2	$k_2 X_3 X_3 - k_{-2} X_4$	$k_2 = 0.01$	$k_{-2} = 0.1$	24
v_3	$k_3 X_4 - k_{-3} X_5$	$k_3 = 1$	$k_{-3} = 0.01$	24
v_4	$V_4 X_5 / (K_4 + X_5)$	$V_4 = 450$	$K_4 = 50$	24
v_5	$k_5 X_5 X_6 - k_{-5} X_7$	$k_5 = 0.06$	$k_{-5} = 0.2$	24
v_6	$k_6 X_7 - k_{-6} X_8$	$k_6 = 1$	$k_{-6} = 0.05$	24
v_7	$k_7 X_8 - k_{-7} X_5 X_9$	$k_7 = 0.3$	$k_{-7} = 0.006$	24
v_8	$V_8 X_9 / (K_8 + X_9)$	$V_8 = 1$	$K_8 = 100$	24
v_9	$k_9 X_9 - k_{-9} X_{10}$	$k_9 = 1$	$k_{-9} = 0.03$	24
v_{10}	$k_{10} X_9 X_{11} - k_{-10} X_{12}$	$k_{10} = 0.214$	$k_{-10} = 5.25$	42
v_{11}	$V_{11} X_{12} X_{13} / (K_{11} + X_{13})$	$V_{11} = 4$	$K_{11} = 64$	42
v_{12}	$V_{12} X_{14} X_{15} / [K_{12}(1 + X_{16}/K_{14}) + X_{15}]$	$V_{12} = 3.5$	$K_{12} = 317$	42
v_{13}	$V_{13} X_{16} / [K_{13}(1 + X_{17}/K_{15}) + X_{16}]$	$V_{13} = 0.058$	$K_{13} = 2200$	38
v_{14}	$V_{14} X_{14} X_{16} / [K_{14}(1 + X_{15}/K_{12}) + X_{16}]$	$V_{14} = 2.9$	$K_{14} = 317$	38
v_{15}	$V_{15} X_{17} / [K_{15}(1 + X_{16}/K_{13}) + X_{17}]$	$V_{15} = 0.058$	$K_{15} = 60$	38
v_{16}	$V_{16} X_{17} X_{18} / [K_{16}(1 + X_{19}/K_{18}) + X_{18}]$	$V_{16} = 9.5$	$K_{16} = 1.46105$	38
v_{17}	$V_{17} X_{19} / [K_{17}(1 + X_{20}/K_{19}) + X_{19}]$	$V_{17} = 0.3$	$K_{17} = 160$	38
v_{18}	$V_{18} X_{17} X_{19} / [K_{18}(1 + X_{18}/K_{16}) + X_{19}]$	$V_{18} = 16$	$K_{18} = 1.46105$	38
v_{19}	$V_{19} X_{20} / [K_{19}(1 + X_{19}/K_{17}) + X_{20}]$	$V_{19} = 0.27$	$K_{19} = 60$	38