A multiscale mathematical model of cancer growth and radiotherapy efficacy: The role of cell cycle regulation in response to irradiation

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Abstract

Background: Prediction of radiotherapy outcomes is usually carried out with the Linear Quadratic model. However, this model does not integrate complex features of tumors, in particular cell cycle regulation.

Methods: In this paper, we propose a multiscale model of cancer growth designed upon the genetic and molecular model established for colorectal cancer evolution. The multiscale model includes identified key genes, cellular kinetic, tissue dynamic and macroscopic tumor evolution, and cycle phase-specific radiosensitivity. We use the model to investigate the role of gene-dependant cell cycle regulation in the response of tumors to irradiation therapeutic protocols.

Results: Simulation results emphasize the importance of tumor growth features and the need to consider regulation factors such as hypoxia as well as tumor geometry and tissue dynamic in prediction and improvement of radiotherapeutic efficacy.

Conclusions: This model provides insights as to how complex biological processes may be coupled to understand colorectal oncogenesis and ultimately will create a better understanding of ways to improve irradiation therapy treatment.

Background

Research into developing mathematical models of cancer growth has been ongoing for many years now. Gompertzian model [1,2], logistic and power functions have been extensively used to describe tumor growth

behavior (see [3] and [4] as examples). These simple formalisms have been also used to investigate different therapeutic strategies such as anti-angiogenic or radiation treatment [5].

Particularly in radiotherapy, the so-called linear-quadratic (LQ) model [6] is still extensively used to study damage to cells by ionizing radiation. Indeed, extension of the LQ model such as the 'Tumor Control Probability' model [7] is aimed at predicting the clinical efficacy of radiotherapeutic protocol applied to cancer patients. Typically, these models assume that tumor sensitivity and repopulation are constant during radiotherapy. However, experimental evidence suggests that cell cycle regulation is perhaps the most important determinant of ionizing radiation sensitivity [8]. It has been suggested that anti-growth signals, such as hypoxia or contact effect, which are responsible for growth fraction to decrease, may play a crucial role in the response of tumors to irradiation [9].

Nowadays, computational power allows us to build mathematical models which can integrate different aspects of the disease, and can be used to investigate the role of complex tumor growth features in the response to therapeutic protocols [10]. In the present study we propose a multiscale model of cancer growth to investigate the role of anti-growth regulation in the response of tumors to radiotherapy. In our model, known key genes of colorectal cancer have been integrated within a Boolean genetic network. Outputs of this genetic model have been linked to a discrete model of the cell cycle where cell radiosensitivity has been assumed to be cycle phase specific. Finally, we use Darcy's law to simulate tumor macroscopic growth within a computational domain.

The model takes into account two key regulation signals influencing tumor growth and consequently therapeutic efficacy. One is hypoxia, which appears when cells lack oxygen. The other one is overpopulation which is activated when cells do not have sufficient space to divide. These signals have been correlated to specific pathways of the genetic model and integrated to the macroscopic scale.

Methods

Oncogenesis is a set of sequential steps in which an interplay of the genetic, biochemical and cellular mechanisms (including gene pathways, intracellular signaling pathways and cell cycle regulation and cell-cell interactions) cause normal cells in a tissue to develop into a tumor. The development of strategies for treatment of oncogenesis relies on the understanding of the pathogenesis at the cellular and molecular level. We have therefore developed a multiscale mathematical model of these processes to study the radiotherapy efficacy. Several mathematical frameworks have been developed to model avascular and vascular tumor growth (see [11–14]. Here we propose a multiscale mathematical model for avascular tumor growth and which is schematically presented in Figure 1. This model can provide a powerful tool for addressing questions of how cells interact with each other and their environment in order to ensure tumor regression during radiotherapy.

Gene level

In colorectal cancer patients, five genes are commonly mutated, namely: APC (Adenomatosis Polyposis Coli), $K\text{-}RAS$ (Kirsten Rat Sarcoma viral), TGF (Transforming Growth Factor), $SMAD$ (Mothers Against Decapentaplegic) and p53 or $TP53$ (Tumor Protein 53). These genes belong to four pathways funneling external/internal signals to cell proliferation or death (see [15] and [16, 17] for more details).

The anti-growth p53 pathway is known to be activated in the case of DNA damage [18, 19] and this is particularly relevant during irradiation [20]. p53 activation can block the cell cycle and induce apoptosis [21, 22]. The $K\text{-}RAS$ gene belongs to a mitogenic pathway which promotes cell proliferation in the presence of growth factors [23]. Activation of the anti-growth pathways TGFβ/SMADs and WNT/APC inhibits cell proliferation. Activation of gene SMAD has been correlated with hypoxia signals [24, 25], while

APC activation through $\beta - catenin$ is known to be linked to loss of cell-cell contact [26–30]. Moreover it has recently been hypothesized that APC mutated cell overpopulation can explain the shifts of normal proliferation in early colon tumorigenesis [31].

We assume that APC and $SMAD$ activation is due to overpopulation and hypoxia signals respectively. Both pathways inhibit cell proliferation. In consequence, APC mutated cells promote overpopulation and $SMAD$ or RAS mutated cells promote hypoxic cells to proliferate. Figure 2 shows the schematic genetic model.

We develop a Boolean model of these pathways in Figure 2. Each gene is represented by a node in the network and the interactions are encoded as the edges. The state of each node is 1 or 0, which corresponds to whether the genetic species is present or not. The states of the node can change in time according to a logical function of its state and the states of these nodes that have edges incident on it [32–34]. The rules governing the genetic pathways are presented in Table 2.

Cell level

We consider a discrete mathematical model of the cell cycle where cycle-phase duration values were set according to literature [35]. In our model the proliferative cycle is composed by three distinct phases, namely: S (DNA synthesis), G_1 (Gap 1) and G_2M (Mitosis). We model the 'Restriction point' R [36] at the end of G_1 phase where internal/external signals, i.e. cell DNA damages, overpopulation and hypoxia are checked [37] (see Figure 3 for a schematic representation of the cell cycle model).

For each spatial position (x, y), we assume that:

- If the local concentration of oxygen was below a constant threshold Th_o and if SMAD was not mutated, hypoxia was declared and leads cells to go to quiescence (G_0) through gene SMAD activation (see Figure 2);
- If the local number of cells was above a constant threshold Th_t and if APC was not mutated, overpopulation was declared and leads cells to go to quiescence (G_0) through gene APC (see Figure 2);
- Otherwise, if the conditions are appropriate, cells enter G_2M and divide, generating new cells at the same spatial position.

Induction of apoptosis through gene p53 activation is discussed later.

Tissue level

We propose to use a model for fluid dynamics to describe the tissue behavior. The macroscopic continuous model is based on Darcy's law which seems to be a good approximation to describe the flow of the tumor cells in the extracellular matrix [38–40]:

$$v = -k\nabla p \tag{1}$$

The media permeability k is assumed to be constant.

We develop a two-dimensional model for the evolution of the cell densities. We formulate mathematically the cell densities in the tissue as advection equations where $n_\varphi(x, y, t)$ represents the density of cells with position (x, y) at time t in a given cycle phase φ. Assuming that all cells move with the same velocity given by equation 1 and applying the principle of mass balance, the advection equations are:

$$\frac{\partial n_\varphi}{\partial t} + \nabla \cdot (v n_\varphi) = P_\varphi \quad \forall \varphi \in \{G_1, S, G_2M, G_0, Apop\}. \tag{2}$$

where P_φ is the cell density proliferation term in phase φ at time t, retrieved from the cell cycle model. Even if not written in equation 2, the global model is an age-structured model as presented in the Simulation technique paragraph. Initial conditions for n_φ are presented in a next section.

Assuming $\sum_\varphi n_\varphi$ to be a constant and adding equations 2 for all phases, the pressure field p satisfies:

$$-\nabla \cdot (k \nabla p) = \sum_\varphi P_\varphi. \tag{3}$$

We set a constant pressure on the boundary of the computational domain.

We assume diffusion equation for the oxygen C with Dirichlet conditions on the edge of the computation domain Ω:

$$\frac{\partial C}{\partial t} - \nabla \cdot (D \nabla C) = -\sum_\varphi \alpha_\varphi n_\varphi \quad \text{on} \quad \Omega \backslash \Omega_{bv} \tag{4}$$

$$C = C_{max} \quad \text{on} \quad \Omega_{bv} \tag{5}$$

$$C_{\partial\Omega} = 0 \tag{6}$$

where D is the oxygen diffusion coefficient which is constant in the computation domain; Ω_{bv} stands for the spatial location of blood vessels, α_φ is the oxygen uptake coefficient by cells at cell cycle phase φ and C_{max} the constant oxygen concentration in blood vessels.

Therapy assumptions

Cell sensitivity depends on cell cycle phases [8]. In the following we assume that only proliferative cells are sensitive to the treatment. We consider the 'single hit' theory assuming that DNA damage is proportional to the irradiation dose.

$$n_{dsb} = R_\varphi d \tag{7}$$

where n_{dsb} is the number of double strand breaks induced by radiation dose d. As said before, the radiosensitivity R_φ has been assumed to depend on the phase of the cell cycle (see Table 3). Based upon radiobiological experiments found in the literature, it has been assumed that the radiosensitivity is constant about 0.2 Gy^{-1} in phase G_1 and G_0, decreases in phase S to 0.2 Gy^{-1}, and then increases to 2 Gy^{-1} during phase G_2.

We set a constant treatment threshold Th_r such as, at any time, if n_{dsb} due to irradiation dose was above Th_r, p53 was activated and cells were labeled as 'DNA damaged cells'. DNA damaged cells are identified at the R point of the cell cycle and are directed to apoptosis. They die and disappear from the computational domain after $T_{Apoptosis}$, i.e., the duration of the apoptotic phase.

The standard radiotherapy protocol used in the simulations consists of a $2Gy$ dose delivered each day, five days a week and can be repeated for several weeks. The radiotherapeutic dose is assumed to be uniformly distributed in the spatial domain.

Model parameters

Cell cycle kinetic parameters have been retrieved from flux cytometry analysis performed on human colon cancer cells [35, 41]. See Table 3 for a summary of the quantitative parameters used.

Computational domain and initial conditions

In our two-dimensional model we study a 8 cm square tissue. We assumed that the domain is composed by five small circular tumor masses, the first one being at the center of the computational domain and the four others towards the corners of the domain. Moreover, the domain is composed by two sources of oxygen disposed at the right and left side of the central cell cluster (see Figure 4).
For the theoretical investigation cells have been uniformly distributed within the five circular tumor masses, that is the cell number is constant in tumors. The number of cells in each phase of the cell cycle is proportional to the duration of such a phase. For instance, G_1 contains twice as more cell than S phase because G_1 phase is twice as long as S. It is important to emphasize that the cell cycle phases are discrete (See the Simulation technique paragraph for details).

According to the radiosensitivity parameters found in literature [42–44], only a fraction of mitotic cells are assumed to be sensitive to the standard $2Gy$ dose.

Simulation technique

The model is fully deterministic. Cell cycle phases duration τ_φ have been discretized in several elementary intervals a, i.e., age; $a \in \{1, \cdots, N_\varphi\}$ where N_φ is an integer such as $\tau_\varphi = dt \times N_\varphi$ with dt the time step of the cell cycle model. Equation for $n_{a,\varphi}$, i.e., density of cell at age a in phase φ can be written:

$$\frac{\partial n_{a,\varphi}}{\partial t} + \nabla \cdot (v n_{a,\varphi}) = P_{a,\varphi}, \tag{8}$$

for $\varphi \in \{G_1, S, G_2M, G_0, Apoptosis\}$ and $a \in \{1, \cdots, N_\varphi\}$ and where $P_{a,\varphi}$ is the cell density proliferation term in phase φ at age a retrieved from the cell cycle model. In the simulations, the intracellular and extracellular conditions were checked for those cells at end of phase G_1. These were used as initial conditions of the gene level model. The genetic model was computed until it reached at steady state (this is usually for 10 iterations).

Since $\sum_{a,\varphi} n_{a,\varphi}$ is constant, summing equations 8 gave the following equation for the pressure field:

$$-\nabla \cdot (k \nabla p) = \sum_{a,\varphi} P_{a,\varphi}, \tag{9}$$

The computer program starts from an initial distribution of cell in each state $\{a, \varphi\}$. The computations are performed using a splitting technique. First we run the cell cycle model for one time step dt, then retrieve new values for $n_{a,\varphi}$ and compute $P_{a,\varphi}$. Pressure is retrieved by solving equation 9 and velocity is computed using Darcy's law (see equation 1).

Since the contribution of the source term has been taken into account by the cell cycle model at the first stage of the splitting technique, equations 8 are solved continuously and without second members :

$$\frac{\partial n_{a,\varphi}}{\partial t} + \nabla \cdot (v n_{a,\varphi}) = 0, \tag{10}$$

which can also be written (using 9:

$$\frac{\partial n_{a,\varphi}}{\partial t} + v \cdot \nabla n_{a,\varphi} = \left(\sum_{a',\varphi'} P_{a',\varphi'} \right) n_{a,\varphi}. \tag{11}$$

This equation is solved by using a splitting technique. Advection parts of equations 11 are solved by the means of sub-cycling finite different scheme computations with time step dt_{adv} being smaller than dt (for

stability reasons).

We set $n_{a,\varphi} = 0$ on the part of the boundary where $v \cdot \nu < 0$ where ν denotes the outgoing normal to the boundary. For the pressure p, we set $p = 0$ on the boundary.

Obviously all the constants could have been functions of cell density or others model parameters.

All simulations (except the ones shown in Figure 7) were run for $320h$ with time step $dt = 1h$ in a discrete computational domain composed by 100×100 elementary spatial units.

Results and Discussion

In this section, we present results obtained by simulation of the mathematical model presented before. The section is divided into three parts. The first one concerns simulations of the model without therapeutic interactions. The second part deals with the interactions between tumor growth and the effect of therapeutic protocols. Finally, we investigate the sensitivity of the results to model parameters and initial conditions. Genetic mutations are simulated by running the model having set constant the Boolean value of particular genes (see Table 2). As the genetic model is run until steady state is reached, simulation of mutated cells growth is equivalent to simulation of cells which are not sensitive to particular anti-growth signals.

In the following, we will call 'cancer cells', cells with at least one mutation. Cells with no mutations are called 'normal cells'.

Gene-dependant tumor growth regulation

Figure 5 shows results of simulated cell colonies growth. According to the model settings, simulated normal cells colony grows up to 10^6 cells and is finally regulated through activation of gene APC due to overpopulation. APC mutated tumor cells are not sensitive to overpopulation and induce an exponential growth until late regulation through $SMAD$ gene activation due to hypoxia. Finally, according to the model parameters, APC and $SMAD/RAS$ mutated tumor cells cannot be regulated at all and thus induce an exponential growth profile.

Simulation results reproduce colorectal cancer evolution [16, 45]. Indeed, APC have been shown to promote the shifts in pattern of normal cell population in early colorectal tumorigenesis, and $SMAD/RAS$ mutations promote evolution from early adenoma to adenocarcinoma.

Features of anti-growth signals and effect on tumor growth

APC-dependant growth regulation

The top picture of Figure 6 shows the evolution of the total and quiescent cell number, when population growth is regulated through activation of gene APC due to overpopulation. Figure 6 shows that the first 100 hours are characterized by oscillations on both curves which slowly disappear and lead to linear growth curves. Indeed, as cell population begins to grow it tends to activate cells gene APC signaling due to overpopulation in the inner part of the tumor masses. This results in a quick increase in the number of quiescent cells, which in turns slow down cell proliferation. Cell advection leads to invasion of new tissues which promotes proliferation and in turns slows down the evolution of the quiescent cell population. These oscillations in the cell population are caused by the balance between the overpopulation signal propagation in the inner parts of the cell clusters and the cell ability to move towards free space tissue. After several proliferation cycles, the recently divided cells move towards free space tissue and are sufficiently numerous to overpopulate the area. This results in a constant proportion of new cells moving to quiescence (see the

late phase of the curves Figure 6). The two snapshots presented at the bottom of Figure 6 shows the spatial distribution of the cells (left), and only mitotic cells (right). Mitotic cells are situated in the outer rims due to overpopulation in the central parts of the clusters.

$SMAD/RAS$-dependant growth regulation

Figure 7 shows the evolution of the total cell number and the number of quiescent cells. In this figure, cells are APC mutated and the growth regulation is controlled by $SMAD/RAS$ signaling, which has been activated due to hypoxia. Before hypoxia, cell population growth is exponential and becomes more linear as the anti-growth signals start.

Figure 8 shows the evolution of the number of co-opted spatial units of the computational domain by the two regulation signals. The overpopulation and hypoxia signal curves can be related to the evolution of the quiescent cells from Figure 6 and Figure 7 respectively. Figure 8 reveals the difference between the hypoxia and overpopulation signaling evolution within the computational domain. The first oscillating growth phase depicted in Figure 6 is caused by the step-by-step evolution of the overpopulation signal activation. Hypoxia activation depicted in Figure 8 appears later and displays only a sharp increase. While overpopulation signal is local - it depends only on the local conditions, the hypoxia signal activation is due to non-local effects. Oxygen absorbed by the cells at a particular position is not available for neighbor cells. This results in a regular signal propagation within the inner parts of the cell clusters as shown in the snapshots of Figure 9. Hypoxia starts from a outer area of the computational domain, that is the more distant areas from the oxygen sources and invades later the central cell cluster where oxygen concentration is the highest.

Influence of gene-dependant growth regulation in the response to irradiation protocols

Simulated irradiation protocols on APC and $RAS/SMAD$ mutated tumor cell

Figure 10 shows the evolution of the number of mutated cells going through apoptosis due to the standard irradiation protocol. In our model the treatment damages a constant fraction of mitotic cells. APC and $RAS/SMAD$ mutated cells are not sensitive to anti-growth signals; they are in hypoxia and overpopulation conditions which leads mitotic cells to grow without regulation. Therefore the number of apoptotic cells increases due to irradiation treatment. However the number of apoptotic cells resulting from one treatment cycle is strictly equivalent to the induced by the previous therapeutic cycle. This is due to difference between the cell cycle duration (33 hours) and the application of the treatment (24 hours).

Simulated irradiation protocols on APC-dependant tumor growth regulation profiles

When cells are sensitive to overpopulation (see growth curves Figure 6), population growth becomes linear after a first oscillating stage. Figure 11 shows the difference in efficacy between two irradiation protocols which are strictly equivalent in terms of the total dose delivered. The first one is the standard protocol (dashed line) where the two doses are delivered with a $24h$ interval. The second one is a heuristic approach where we optimized the second dose delivery considering cell cycle regulation; the second treatment is given when the number of the mitotic cells reaches a maximum. The first treatment application decreases the number of tumor cells. Note that the dotted line in Figure 11 is hidden by the continuous line. This also occurs in the second treatment of the heuristic protocol. However the second treatment delivered without taking into account growth regulation, i.e., standard scheduling, results in a very poor efficacy (see Figure 11).

Simulated irradiation protocols on APC-mutated ($RAS/SMAD$-dependant) tumor growth regulation profiles

Figure 12 shows the evolution of the irradiation target cell population fraction, i.e., mitotic fraction over time without irradiation before and after the activation of the hypoxia signal. As soon as the hypoxia appears, the

mitotic fraction collapse. Table 1 shows the difference in simulated efficacy between two equivalent protocols in terms of total dose. The first is the standard protocol where the $2Gy$ treatment are given daily, 5 days a week and for 2 weeks with total dose is $20Gy$. The second is an heuristic treatment in which all the 10 doses of $2Gy$ are given before the hypoxia signals appear. Part of the standard treatment is delivered while tumors became hypoxic (mitotic fraction fell down), which results in efficacy decreasing. On the contrary all the 10 doses of the heuristic treatment are delivered before hypoxia which leads to a better efficacy.

Sensitivity to model parameters and initial conditions

We study the potential influence of the choice of parameters values on the model's results. Most critical parameters that must be accounted for include:

- cell-specific radiosensitivity parameters (α_φ));
- anti-growth signals i.e., hypoxia and overpopulation, activation thresholds above which cells go into quiescence (Th_o and Th_t);
- initial conditions, i.e., initial number of cells and spatial configurations of oxygen sources.

Treatment protocol efficacy depends directly on cell-specific radiosensitivity parameters. Figure 13 compares the total cell number evolution over time when standard treatment protocol is applied. Model simulations shows significant efficacy to the standard treatment when the radiosensitivity parameters make cells in G_1 phase become radiosensitive. APC, and $SMAD/RAS$ activation which leads cells to go to quiescence is decided upon the two threshold parameters Th_t and Th_o. Increasing Th_t results in delaying the overpopulation signal, while increasing Th_o brings hypoxia activation forward.

Decreasing the initial number of cells is equivalent to increasing Th_t, while decreasing the number or the initial concentration of the oxygen sources is equivalent to increasing Th_o. The initial configuration of tumor cells and oxygen sources is important on hypoxia signal spatial propagation. Indeed, Figure 9 shows a particular hypoxia propagation in the cellular tumor masses which is correlated with the locations of the oxygen source. Since Th_t and Th_o are constants we may be changing the spatial configuration of initial cell population, e.g. different number and locations of cell clusters and of oxygen sources might not produce different qualitative results.

Finally Figure 14 shows the evolution of overpopulation signal over time when cells in initial cell clusters are distributed uniformly and randomly. The step by step evolution of overpopulation activation is softened but still existing when cells are randomly distributed within the initial tumor masses.

Conclusions

We presented a multiscale model of cancer growth and use it to predict the qualitative response to radiotherapy. The mathematical framework includes a Boolean description of a genetic network relevant for colorectal oncogenesis, a discrete model of the cell cycle and a continuous macroscopic model of tumor growth and invasion. Sensitivity to irradiation depends on cell cycle phases and DNA damages are proportional to the radiation dose. Anti-growth regulation signals such as hypoxia and overpopulation activate genes $SMAD/RAS$ and APC respectively and inhibit proliferation through the cell cycle regulation.

Simulation results show the different features of the anti-growth signal activation and propagation within the tumor (see Figure 8). Overpopulation signal mediated by APC gene is at first evolving step-by-step which induces an oscillating growth profile due to a balance between proliferating and quiescent cells (see Figure 6). Due to non-local effect, the hypoxia signal mediated by genes $SMAD/APC$ appears later but quickly develops within the tumor masses and leads the mitotic fraction to collapse (see Figures 11 and 14).

These features make the evolution of the number of quiescent cells and thus the efficacy of irradiation protocols to depend on the type of anti-growth signals the tumors undergo. Figure 11 and Table 1 show that efficacy could be improved without increasing doses but by planning schedules by considering tumor growth features through cell cycle regulation.

The proposed framework emphasizes the significant role of gene-dependant cell-cycle regulation in the response of tumors to radiotherapy. Clinical studies have recognized p53 status as a major predictive factor for rectal cancer response to irradiation. Nevertheless some results encourage open investigation to other different factors [46]. In particular the importance of macroscopic factors such as hypoxia and tumor volumes have been suggested [47]. The present modeling framework integrates these factors through cell cycle regulation and allows to consider other factors of the genetic, cellular or tissue scale.

Some simplistic modeling assumptions must be discussed. We chose a continuous approach which provides density of cells rather than actual cell number. This is realistic only if any one region of interest is very large and will not be appropriate in the early stage of tumor growth or in other conditions such as angiogenesis where microscopic environment is well known as the major determinant of cancer progression. Also we did not modeled cell shape which has been shown to be important for a correct description of growth control processes [48]. Individual based models of cell movement, e.g. Potts model [49, 50] and the Langevin model [51] would constitute an improvement of the present work. Finally, the reduction to the two dimensional problem is far from being realistic since diffusion-limited processes extensively used in this paper depends on the dimension. Also it is worth mention that a three-dimensional tumor growth model could bring into perspective new factors in its growth dynamics. Finally, improvements will have to be performed to realize a computational domain which simulates a virtual colonic crypt if we want to predict therapy efficacy for colorectal cancer.

Clearly the study is not aimed at predicting quantitatively the effect of a therapeutic protocol. Nevertheless the theoretical analysis performed allows us to raise some interesting facts on the role of anti-growth regulation signals and potentially of particular genes on standard protocol efficacy.

Nowadays efforts are made to optimize the LQ model by taking into account multi factors such as tumor volume or repopulation between treatment cycles [52]. The multiscale model of radiotherapy efficacy we propose may serve as a theoretical basis for optimizing a predictive modeling tool for radiotherapy outcomes.

Authors contributions

BR designed the mathematical multiscale model and simulated it to investigate the role of cell cycle regulation in response to irradiation treatment protocols. TC designed the macroscopic level. He implemented advection-diffusion equations and contributed in linking the sub-models together. SS elaborated the genetic boolean network model of colorectal oncogenesis and its implementation. He also supervised manuscript revision.

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Figures

Figure 1 - Multiscale nature of the model

Schematic view of the multiscale nature of the model composed by four different modules. The genetic module integrates the main genes of colorectal cancer evolution within a Boolean network and results in cell cycle regulation signals. These signals are treated within the cellular module which determines a ratio between proliferation and death. This ratio is used as an input of the macroscopic model with compute the cells spatial distribution within the computational domain. Cells number and spatial configuration determine the activation of the anti-growth signals which feedback as inputs of the genetic model. Finally, irradiation induces DNA breaks which activate p53 gene from the genetic network.

Figure 2 - Cell proliferation and death (genetic regulation) for colorectal cancer

The genetic model with regulation signals as inputs. p53 is activated when DNA is damaged and leads the cell to apoptosis. $SMAD$ is activated through $TGF\beta$ receptors in case of hypoxia and inhibits the cell's proliferation. Overpopulation inhibits the cell's proliferation through activation of gene APC. RAS promotes cell's proliferation through growth factors receptors when sufficient oxygen is available for the cell, that is, no hypoxia. This flow chart was developed from knowledge available from bibliographic resources [15, 16] and from the Knowledge Encyclopedia of Genes and Genomes [53, 54].

Figure 3 - Diagram of the cell cycle model

In the discrete model of the cell cycle, cells are progressing step by step in the phases of the cell cycle. The proliferative cycle is composed by three phases: G_1, S, and G_2M. At the end of the G_2M phase, cells divide and new born cells begin their cycle in G_1. At the last step of phase G_1, we modeled the restriction point R, where DNA material and external conditions are checked (overpopulation and hypoxia). If overpopulation and/or hypoxia occurs, genes APC and/or SMAD are activated respectively and lead cells to G_0 (quiescence). Cells at the last step of the quiescent phase are stocked. These cells can go back in the proliferative cycle (at the first step of phase S) if the external conditions allow it. DNA damages can activate $p53$ which leads cells to the apoptotic phase. Cells at the end of the apoptotic phase die and disappear from the computational domain.

Figure 4 - Initial conditions
Illustration of the two-dimensional computation domain for the model simulations with initial cells spatial configuration. The domain is composed by five cell clusters and two blood vessels.

Figure 5 - Cell population growth
Cell population growth (log plot) over time according to three different genetic profiles. Normal cells (black diamonds), APC mutated cells (dashed line), and $APC + RAS/SMAD$ mutated cells.

Figure 6 - APC-dependant growth regulation
Top: Evolution of the number of quiescent cells and total number of cells over time (log pot). Cell population is regulated through gene APC activation due to overpopulation. Total cell number (continuous line) and number of quiescent cells (dotted line).
Bottom: Snapshots of cells within the computational domain during simulation ($t = 100h$).
Left: Total cell number; Right: Mitotic cells compose only the outer rim of the tumor masses. Cells composing the inner parts are quiescent through APC activation due to overpopulation.

Figure 7 - $SMAD/RAS$-dependant growth regulation
Evolution of the number of quiescent cells and total number of cells over time (log pot). APC mutated cell population is regulated through SMAD/RAS activation due to hypoxia. Total number of cells (continuous line) and number of quiescent cells (dotted line)

Figure 8 - Anti-growth signals
Number of co-opted spatial units of the computation domain by the two regulation signals. The two curves show the activation of the hypoxia (continuous line) and overpopulation (dashed line) signals over time. The vertical axis represents the number of elementary spatial units of the computational domain.

Figure 9 - Evolution of the spatial distribution of the mitotic cells
Hypoxia signal propagation within the tumor masses (from top-left to bottom-right). Inner black areas are cells in quiescence due to $SMAD/RAS$ activation through hypoxia.
Top, Left: Spatial distribution of mitotic cells at time $48h$; Top, Right: $112h$; Medium, Left: $168h$; Medium, Right: $224h$; Bottom, Left: $290h$; Bottom, Right: $336h$.

Figure 10 - Apoptotic activity
Number of cells in the apoptotic phase over time when applying the standard radiotherapeutic protocol: 2Gy daily. Vertical black arrows indicate treatment delivery times. Note that apoptotic activity appear with a time delay respect to treatment delivery. This is the time needed for the G_2M DNA-injured cells to reach the restriction point of the cell cycle (21 hours according to the model parameters).

Figure 11 - Comparison of two radiotherapeutic protocols
Top: Total cell number in response of a standard therapeutic scheduling, i.e., 2 Gy applied twice with a 24 hours interval, or in response to an heuristic scheduling. Note that for the 40 first hours, the dotted line is hidden by the continuous line since the first treatment dose is applied at the same time; Bottom: Evolution of the number of apoptotic cells due to irradiation protocols. First treatment induces the same number of

apoptotic cells. The second treatment effect of the standard protocol is negligible (black diamonds around time $50h$) respect to the heuristic approach (white diamonds pick at $40h$). Treatment delivery times are symbolized by vertical arrows. White for the standard scheduling and black for the heuristic approach.

Figure 12 - Simulated mitotic fraction evolution of APC-mutated cells over time without irradiation
The vertical dashed line indicated the time where hypoxia signal has been activated and cells go to quiescence through $SMAD/RAS$ signaling.

Figure 13 - Effect of radiosensitivity parameters in treatment efficacy
Evolution of the total cell number over time with the previous radiosensitivity parameters (continuous line), and with new parameters so that cells in phase G_1 are sensitive to the $2Gy$ treatment dose.

Figure 14 - Effect of cell distribution within the initial cell clusters on overpopulation
The vertical axis stands for the number of elementary spatial units of the computational domain.

Tables

Table 1 - Apoptotic activity
Apoptotic activity induces by two $20Gy$ radiotherapy protocols applied on APC-mutated tumor cells.

Apoptotic activity				
	Total dose (Gy)	Scheduling	Apoptotic fraction -Mean- (%)	Apoptotic fraction -Max- (%)
Standard protocol	20	2Gy daily	2.59	4
Heuristic	20	2Gy 10× before hypoxia	3.14	4.25

Table 2 - Genetic model
Boolean (logical) functions used in the genetic model depicted Figure 1. For APC, $SMAD$, and RAS, Boolean values are set to 0, 0, and 1 respectively when genes are mutated.

Boolean model	
Node	Boolean updating function
APC^t	$APC^{t+1} = \begin{cases} 1 & \text{if Overpopulation signal} \\ 0 & \text{otherwise} \end{cases}$ $APC^{t+1} = 0$ if mutated
βcat^t	$\beta cat^{t+1} = \neg APC^t$
$cmyc^t$	$cmyc^{t+1} = RAS^t \wedge \beta cat^t \wedge \neg Smads^t$
$p27^t$	$p27^{t+1} = SMADs^t \vee \neg cmyc^t$
$p21^t$	$p21^{t+1} = p53^t$
Bax^t	$Bax^{t+1} = p53^t$
$SMAD^t$	$SMAD^{t+1} = \begin{cases} 1 & \text{if Hypoxia signal} \\ 0 & \text{otherwise} \end{cases}$ $SMAD^{t+1} = 0$ if mutated
RAS^t	$RAS^{t+1} = \begin{cases} 1 & \text{if no Hypoxia signal} \\ 0 & \text{otherwise} \end{cases}$ $RAS^{t+1} = 1$ if mutated
$p53^t$	$p53^{t+1} = \begin{cases} 1 & \text{if DNA damage dignal} \\ 0 & \text{otherwise} \end{cases}$ $p53^{t+1} = 0$ if mutated
$CycCDK^t$	$CycCDK^{t+1} = \neg p21^t \wedge \neg p27^t$
Rb^t	$Rb^{t+1} = \neg CycCDK^t$

Table 3 - Table of parameters

Table of numerical parameters used for simulations.

Model parameters				
Parameter	Description	Unit	Value	Reference
T_{G_1}	Duration of G_1 phase	h	20	[35, 41]
T_S	Duration of S phase	h	10	[35, 41]
T_{G_2M}	Duration of G_2M phase	h	3	[35, 41]
T_{G_0}	Duration of G_0 phase	h	5	Estimated
$T_{Apoptosis}$	Duration of the apoptotic process	h	5	Estimated
C_{max}	Oxygen in blood	mlO_2	10^{-2}	Estimated
α_φ	Oxygen consumption in phase φ	$mlO_2 s^{-1}$	$5 - 10$ $\times 10^{-15}$	Estimated
Th_o	Hypoxia threshold	$cell^{-1}$	5×10^{-15}	Estimated
Th_t	Overpopulation threshold	$cell$	2000	Estimated
R_φ	Cell Radio--sensitivity in phase φ	Gy^{-1}	0.2 - 2	[42–44]
k	Media permeability	m^2	0.2	Estimated