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Model of avascular tumor growth and response to low dose exposure

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Abstract. A single level cellular automata model is described and used to simulate early tumor growth, and the response of the tumor cells under low dose radiation affects. In this model the cell cycle of the population of normal and cancer cells is followed. The invasion mechanism of the tumor is simulated by a local factor that takes into account the microenvironment hardness to cell development, in a picture similar to the AMTIH model. The response of normal and cancer cells to direct effects of radiation is tested for various models and a model of bystander response is implemented.

1. Introduction

The main goal of radiotherapy is the targeting of malignant cells with ionizing radiation to kill them without affecting the neighboring normal tissue. In this sense computational simulations can help to increase the effectiveness of radiotherapy. To achieve reliable results the response of cells to direct and indirect effects of ionizing radiation should be taken into account, and the growth and invasion of tumors should be accurately reproduced, simultaneously.

Mathematical modeling of tumor growth and tumor response to radiation has increased our knowledge in this field. In this path several models have been proposed to simulate early avascular tumor growth [1, 2]. The discrete approach of cellular automata have been developed using one[3], two [4] or more levels [5, 6]. The main reason behind the multi level cellular automata simulation rests in the disparity in characteristic diffusing time of the chemical species involved in the cells metabolic processes. Nevertheless a one level cellular automata model is able to simulate observed growth behavior of avascular tumors with a low number of parameters [3]. This simplified model requires more attention in the interpretation and meaning of the parameters involved, but his simplicity gives some insights in the underlying invasion mechanisms. The Acid mediated tumor invasion hypothesis (AMTIH) is based on the acidification of the microenvironment that the proliferating cancer cells generate [5]. The *growing potential* factor used by [3] can be interpreted as a crude approach of the AMTIH mechanism.

In recent years the radiobiological attention has been focused in the observed radiation induced bystander effect (RIBE). The development of microbeam irradiation techniques [7] have expanded our knowledge in this phenomenon. In this work we proposed a new model of CA that can reproduce the expected growing behavior of avascular solid tumors and, simultaneously reproduce the low dose region response of cells to ionizing radiation.

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2. Model

The model is set as a bidimensional grid with constant grid size, and one to one correspondence between physical cells and sites. Each grid element is represented by a vector state: $(ns, np, g)_{i,j}$. Where i and j are the position coordinates of the cell in the grid . ns is the cell state, that can be in one of four possible states: empty, normal, cancer or necrotic. np is the cell phase in his normal cycle, the normal and cancer cells can be in one of the four possible states (i.e. G_1 , S , G_2 M). And g is the growth factor. This later value is intended to represent the influence of the medium to cellular viability as a global weight that takes into account all the chemical fields involved in cellular development. In this sense the g factor is interpreted as single factor enclosing the acidification of the microenvironment by lactose production of active cells and the depletion of useful vital resources (i.e. glucose and oxygen) in the neighborhood of the cell. Because of the correlation between waste products and consumption of resources, these two fields are treated in one single factor. Normal cells are autoregulated and are less active than the cancer ones, so his g factor remains in the basal value $g_0 = 10^{-H_0}$ (with H_0=7.4), but the cancer cells with increased activity consume more resources and originates a rise in the waste products, so the sites occupied by cancer cells continually increases the local g.

2.1. Cell cycle

The CA cycle is set to last one hour. And the cell cycles comprised: gap 1 (G_1), Synthesis (S), gap 2 (G_2) or mitosis (M). The last three phases have constant duration and the G_1 phase duration is probabilistic. Because of the deterministic nature of the cell evolution beyond G_1 phase, the probability of transition from G_1 to S phase is identified with the probability of mitosis.

The probability of mitosis is calculated as a global measure of the number of cancer and normal cells, according to a logistic like equation:

$$p_m = p_0 \left(1 - \frac{N_t}{K}\right) \tag{1}$$
$$N_t = 2N_c + N_n$$

Where: p_m is the mitotic probability, p_0 is the basal probability and K is the carrying capacity that is set as the total number of cells in the grid. To take into account the greater activity of the cancer cells, for which the glucose consumption rate is observed to be of the order of 5 to 10 times the normal cells consumption rate [8], the total number of cancer cells N_c is double weighted, with respect to the number of normal cells N_n. This mechanism depends on the dynamics and on the number of cancer and normal cells, but in order to enhance the effect of this global feedback inhibition mechanism, a local influence of the cancer cells is considered through the g factor. In every site the mitotic probability is obtained by the expression:

$$p_{i,j} = p_m . e^{(H_o - H_{i,j})} \tag{2}$$
$$H_{i,j} = -log(g_{i,j})$$

Where i, j are the index numbers of the site in the grid, and $g_{i,j}$ is the growth factor of the i,j site.

2.2. Cellular automata rules

All the elements of the grid are updated in each cycle of the simulation, in random order to avoid artifacts that can arise from the scanning method. Each cell can change its vector state according to the next local rules:

(i) Normal cells: the mean growth factor $\bar{g}$ is calculated in the Moore neighborhood [9] of the cell $\bar{g} = \sum_{i,j} g_{i,j}/N$, with i, j index changing in all the neighbor sites. If the value $H_{i,j} = -log(\bar{g})$ is greater than the surviving threshold value for normal cells (H_n) then the cell remains in normal state, with $g_0 = 10^{-H_0}$. Otherwise the cell is incapable of sustain his vital activity and becomes unviable, so the site changes to empty site with $g = g_0$. If the local $H_{i,j} > H_n$ and there is at least one empty site in the Moore neighborhood of the cell, then his phase state is increased.
The phases in the cycle have constant duration with the only exception of the G_1 phase that has a minimum duration, but the transition to S phase is probabilistic, with the probability given by equations 1 and 2. If there is any free site then the normal cell is autoregulated and remains in G_1 state and keeps his local value of g_0. When the cell reaches the end of his cycle, and there is at least one empty site, then mitosis occur and one randomly chosen empty site is used for replication. In mitosis the mother cell is eliminated and one of the daughters is placed in the mother site, and the other in the empty adjacent site, both of them at the beginning of G_1 state.

(ii) Cancer cells: if there is, at least, one neighbor site in *empty* state, then the cancer cell cycles in the same manner as the normal cells. Mitosis proceeds in the same way as in the normal cells, previously described. The local g value is increased in every loop of the simulation. In order to take into account the nutrients depletion, and the related waste production rate, the local $H_{i,j}$ is increased. The increment is given by: $\Delta H_c = \Delta H_{c0}.p_m$. Where p_m is given by equation 1, and ΔH_{c0} is the maximum increment allowed for cancer cells.
If all the neighbors are occupied and the local $\bar{g}$ is lower than a given threshold H_c then the cell becomes necrotic. In the same situation of all the neighborhood filled when the local $\bar{g}$ reaches a value below a threshold H_q but is above H_c, then the cells enter in a quiescent state and his increment of g is lowered to $\Delta H_q = \Delta H_q/100$ [5].

(iii) Necrotic cell: In this state the local $g_{i,j}$ remains constant (his value depends on the g-value of the cell that originated the necrotic site). If a necrotic cell has one or more neighbors that are empty site, then it can change to empty site with probability $1/2T$, where T is the total period of cell cycling.

(iv) Empty site: an empty site can be filled with mitotic cancer or normal cells in the mechanisms described above. In the case of competing normal and cancer cells, the cancer cells have priority to fill the empty site.

2.3. Radiation response

In order to take into account the hyper radio-sensitivity (HRS) effects and the increased radio resistance above 0.5 Gy [10] two set of parameters in the lineal quadratic model are used. The values are the same as used by [11] for glioma cells and are reproduced here only for completeness. Each phase of the cells have his surviving curve given by the parameters of the table 1.

Table 1. Parameters used to calculate the surviving fraction of irradiated glioma cells from reference [11]. Mean value and standard deviations refer to threshold that triggers repair process.

Phase	α_s	α_r	β	Mean	Standard deviation
G_1	0.615	0.03	0.038	0.508	0.33
S	6.803	0.266	-0.012	0.113	0.264
G_2/M	0.329	0.048	0.043	0.429	0.03

The surviving fraction is computed with:

$$Sf(d) = e^{-(\alpha_\mu + \beta d)d} \tag{3}$$

Where the μ index refer to the low or high dose response. The region of dose response depends on the particular threshold value that every cell in the population has. This threshold is distributed among the population with mean and standard deviation given in table 1. As a consequence of radiation damage the cells affected with the probability given by equation 3 can be killed with two different mechanisms: a) if the DNA damage is severe enough the cell suffer apoptosis in a process known as Radiation induced interphase dead (RIID), in this case the cell changes to empty site immediately, with probability 0.1 . b) The cell DNA can be affected in a way that his mitotic daughters are not viable any more, this happens in Radiation induced mitotic dead (RIMD) with 0.9 probability. To account for the later mechanism of cell killing an extra set of parameters should be added to the vector state of each cell. The cells marked to be killed in RIMD are treated as usual in the simulations, but at the end his M phase booth daughter cells suffers apoptosis and are replaced for empty sites in the grid.

2.4. Bystander

Non-targeted effects of ionizing radiation are caused by intercellular chemical signaling that diffuse in the media; they include production of DNA damage and alterations in cell apoptosis, differentiation, senescence or proliferation. The bystander phenomenon results from signaling molecules that rapidly propagate from irradiated cells and decrease in concentration as distance increases [12]. The bystander effect is modeled as a global effect that can kill any cell in the grid that have survived, or have never been exposed to radiation. The signal variation of intensity with distance is not modeled in this work, because of the distances involved in our simulations. Exposed cells becomes sources of the bystander signal, that can last in the medium 4 days in our simulations [13, 14]. If a cell survives to the first bystander effect it becomes immune to the signal. This mechanism was simulated according to the next rules:

(i) Cells that have been directly exposed to radiation and survives can emit an adimensional bystander signal with probability:

$$p_b = 1 - e^{-d/d_l} \tag{4}$$

with d_l chosen to be 0.1 Gy in accordance to the observations from [7] for 278 eV C_k x-ray beam over V79 cells. The bystander signal is approximately linearly dependent from the dose and saturates at 0.3 Gy of exposure.

(ii) The proportion of cells exposed to the signal that were killed is calculated by the expression:

$$df(s) = df_{max}(1 - e^{10.s(t)}) \tag{5}$$

where s(t) is the concentration of the signal in the medium and df_{max} is the maximum proportion of killed cells, for the current simulations this parameter where chosen to be 0.1 for all cell phases.

(iii) The cells exposed to the bystander signal that survives becomes immune to it. But can become secondary sources of bystander signal with probability given by equation 4.

3. Results

The parameters for the model were chosen in accordance with the typical tumor growth observations of spheroids in vivo and in vitro. The cell cycle duration widely vary between different kind of cells, for the purposes of this work we consider a typical duration of cell cycle in the order of experimental observations [15]. G_1 duration is of 12 hours, S phase duration is of 7

hours, G_2 phase duration is of 4 hours and M phase last 1 hour, to give a 24 hs cell cycle period. The same cycle phases duration is used for normal and cancer cells [15] as a valid simplification for the purposes of this work .

3.1. validation of the model and parameters: tumor growth
The general model for the ontogenetic growth of living organisms of West et al [16, 17] have been successfully translated to the growth of solid malignant tumors in-vitro and in-vivo [18]. This general or *universal* model rests in the simple expression:

$$r = 1 - e^{-\tau} \tag{6}$$

Where r and τ are the adimensional variables:

$$r = \left(\frac{m}{M}\right)^{0.25} \qquad \tau = \alpha.r_0.t - ln(1 - r_0) \qquad r_0 = \left(\frac{m_0}{M}\right)^{0.25}$$

Where m_0 is the initial tumor mass and M is the final mass of the tumor. In this work we are assuming the following equivalence between mass rate and cell number rate as a valid approximation:

$$r = \left(\frac{n}{N}\right)^{0.25} = \left(\frac{m}{M}\right)^{0.25}$$

Where n is the number of cancer cells and N is the asymptotic final number of cancer cells. The parameter α is approximately constant in a given taxon and proportional to the organism metabolic rate across taxa [16]. In the case of solid tumors α is supposed to be related with tumor particular characteristics as its invasion ability [18]. This parameter is obtained from the linear regression:

$$ln(1 - r) = t.\alpha.r_0 - ln(1 - r_0) \tag{7}$$

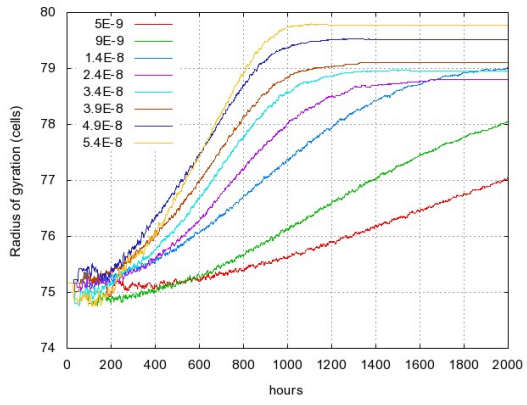

Figure 1. Radius of gyration of tumor for different values of ΔH_{c0}. The reference of colours is shown in the picture.

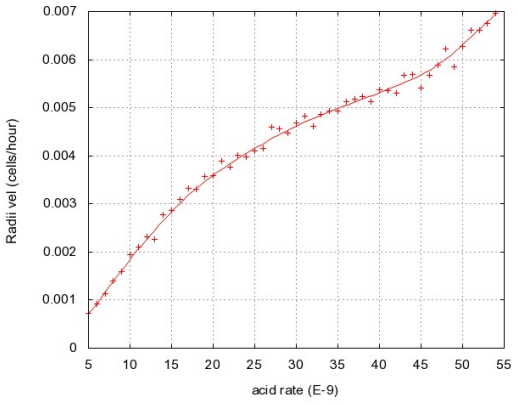

Figure 2. Mean velocity of the radius of gyration vs ΔH_{c0}. Simulations are the red cross and a smoothing line is set as visual reference.

This growth behavior is used as selective criterion for adjusting the parameter ΔH_{c0}. This parameter is directly related with the growing rate of the radius of gyration of the tumor in

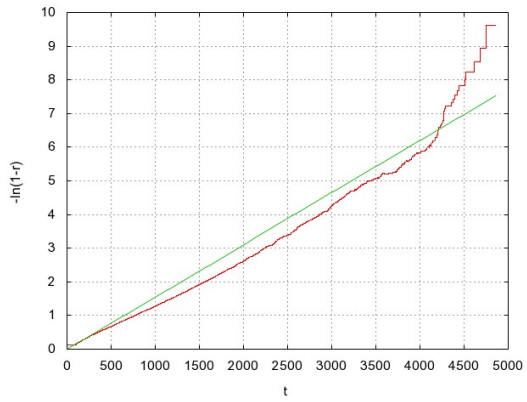

Figure 3. Linear regression (green line) of the reduced variables r and τ (red line) to obtain the α parameter of equation 7.

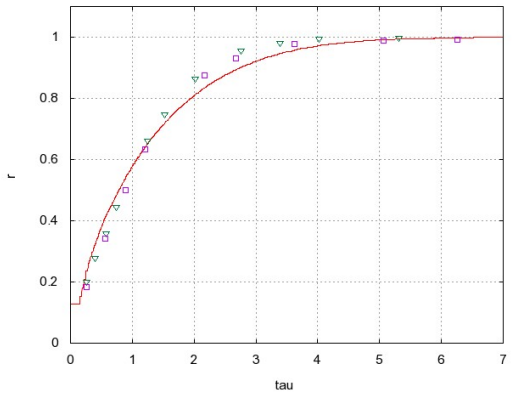

Figure 4. Relation of reduced variables r and τ of the present simulations. Experimental *in vivo* data from reference [19] (green $\bigtriangledown$) and [20] (purple $\square$) (extrapolated values from [18]).

our simulations (see figure 1 and 2). The radius of gyration is defined as the square root of the second moment of the spatial distribution.

The best value for the parameters where chosen to be $\Delta H_{c0} = 8.10^{-9}$ and the values for quiescent threshold $H_q = 6.04$. The values of the remaining parameters where set according to the pH of serum acid concentration and threshold surviving values of pH of normal and cancer cells [5] $H_0 = 7.4$, $H_c = 6.0$ and $H_n = 7.1$. These values where used in all the present simulations.

3.2. validation of the model and parameters: surviving fraction of V79 cells

In order to test our simulations against available experimental data the radiation response of V79 cells to 250 KeV X rays were simulated with the multitarget model:

$$df(d) = \left(1 - e^{-d/d_0}\right)^n \tag{8}$$

Where the parameters are n=2 , and $d_0 = 1/0.44$ [21]. Simulations where carried out with randomly distributed cells in an empty grid. The entire grid where irradiated once and the cells where allowed to cycle during 72 hours. Then the surviving fraction were calculated as the rate $s_f = n_s/n_0$ of the surviving population n_s against the control unirradiated population n_u. Simulations were run with and without bystander response. The increased surviving fractions of the series without Bystander effect separates the simulated results from the experimental data (see figures 5 and 6).

4. Discussion and concluding remarks

The present model is the first one level cellular automata model that can reproduce cellular cycling and tumor invasion mechanisms of normal tissue. A simplified model of tumor invasion through resource depletion and acidification of microenviroment is proposed, inspired in the AMTIH hypothesis. Is expected that original vascularization of invaded tissue and neovascularization of growing tumor were crucial for tumor growth in more advanced stages, so the present model should be limited to avascular tumor growth stages. The response of cells to low radiation exposure is well reproduced and a simpler bystander mechanism is proposed. Further investigations and experiments are needed to fully incorporate RIBE for

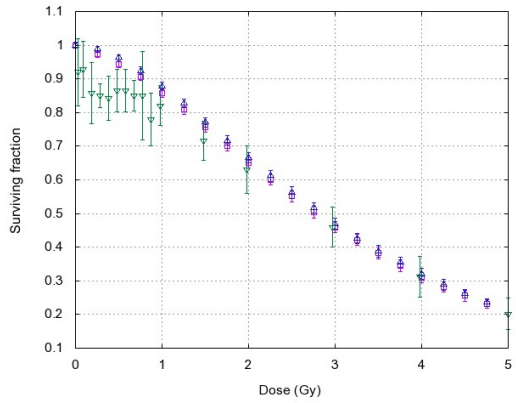

Figure 5. Surviving fraction of simulated V79 cells cultured in vitro, irradiated with 250 KeV x-rays using the multitarget model (eq. 8). With RIBE : purple □, without RIBE: blue △. Experimental values from [22] (green ▽).

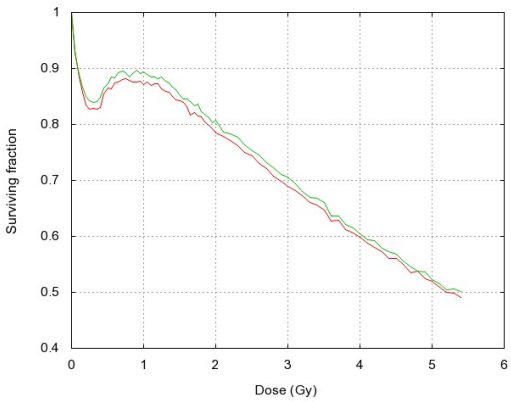

Figure 6. Surviving fraction of simulated Glioma cells using equation 3 with parameters given in table 1. With RIBE (red line) and without RIBE results (green line).

different radiation types and modulation of Bystander effect with cell cycle. The model allows to taking into account the cell cycle response to non direct and direct radiation effects.

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