Chemotherapy in heterogeneous cultures of cancer cells with interconversion

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Abstract

Recently, it has been observed the interconversion between differentiated and stem-like cancer cells. Here, we model the *in vitro* growth of heterogeneous cell cultures in the presence of interconversion from differentiated cancer cells to cancer stem cells, showing that, targeting only cancer stem cells with cytotoxic agents, it is not always possible to eradicate cancer. We have determined the kinetic conditions under which cytotoxic agents in *in vitro* heterogeneous cultures of cancer cells eradicate cancer. In particular, we have shown that the chemotherapeutic elimination of *in vitro* cultures of heterogeneous cancer cells is effective if it targets all the cancer cell types, and if the induced death rates for the different subpopulations of cancer cell types are large enough.

1 Introduction

Stem cells are cells with the ability of generating mature cells of a tissue by differentiation, having also the ability of self-renewal, persisting during the life time of an animal, [8]. Stem-like cells have been identified in several tumours, making them particularly aggressive, [6, 1].

Recently, it has been observed that non-stem cancer cells give rise to cancer stem cells (CSC) *in vivo* and *in vitro*, [3, 5]. This has been observed in cultures of breast cancer cells, and the rates of interconversion between differentiated cells and stem-like cells have been measured, [5]. As noted

by Chaffer *et al.*, [3], this interconversion can annulate the effectiveness of clinical treatment of cancer by targeting only CSC.

Chaffer *et al.*, [3], and Gupta *et al.*, [5], considered several samples of epithelial breast cancer cells divided into three subpopulations: luminal cells, basal cells and stem-like cells. Then, they have grown *in vitro* cultures of these cells, initiated from different proportions of initial subpopulations. After several hours of cell growth in nutrient-rich media, the cell culture subpopulations converged to constant cell number proportions. In the presence of nutrients, cells continue to grow, with constant relative cell-state proportions. With the help of cell proliferation mathematical models, theses authors estimated the rates of transition between cell states and have shown that differentiated cell types, basal and luminal, can convert to stem-like cells after self-renewal.

The experimental data has been analysed with simplified cell proliferation models. Chaffer *et al.*, [3], developed a continuous time simplified model, where they have considered a non zero rate transition from a differentiated cell state to a stem cell state. Gupta *et al.*, [5], used a discrete Markov chain model for cell-state interconversion between stem (s), basal (b) and luminal states (l). For example, in one of the cultures of Gupta *et al.*, [5], (SUM149), the calculated transition probability between cell-states are: $p_{s\to s} = 0.61$, $p_{b\to b} = 0.90$, $p_{l\to l} = 0.99$, $p_{s\to l} = 0.30$, $p_{l\to s} = 0.01$, $p_{s\to b} = 0.09$, $p_{b\to s} = 0.01$ and $p_{b\to l} = 0.08$. From these quantitative values, it follows that CSC can regenerate from non-CSC.

Our goal here is to model the growth of heterogeneous cell cultures in the presence of interconversion between differentiated cancer cells to CSC cells, and to evaluate the efficacy of chemotherapeutic strategies. There are several mathematical modelling strategies to analyse the growth of cancer cells. Some of the models focus on the growth rate and form of tumours, others are centred on the kinetics of *in vitro* cultures of tumour cells. From the therapeutic point of view, *in vitro* tumour cells growth gives a first approach to estimate the kinetic rate parameters for *in vivo* tests, [2].

This paper is organised as follows, in section 2 we derive a proliferation model for *in vitro* cultures of cells with two subpopulations (stem-like cells and non-stem or differentiated cells). This model includes not only all the possible rates of transitions between the two subpopulations (interconversion), but also includes the effect of cytotoxic agents. Then, we analyse the growth rate of the population of cell, showing that in the presence of resources and growth factors, the cell subpopulations grow indefinitely with fixed proportion between subpopulations. In this model, nutrients are considered freely available. In section 3, we analyse the inhibition of growth of CSC and of differentiated cells by the action of cytotoxic external agents. Fi-

nally, in section 3 we discuss the main conclusions of the paper. To simplify the exposition, all the formal proofs were moved to Appendix A.

2 Modelling heterogeneous cell growth

We consider two types of cells in an *in vitro* culture. Stem-like cells denoted by S and differentiated cells denoted by D. From the modelling point of view, these cell can be normal cells or cancer cells. In *in vitro* cultures and in the presence of nutrients, these cells duplicate by mitosis and can self-renew in different cell types. We describe the process of cell duplication in the presence of nutrients by the kinetic diagrams,

$$\begin{array}{l} \mathrm{A} + \mathrm{S} \xrightarrow{k_1} 2\mathrm{S} \\ \mathrm{A} + \mathrm{S} \xrightarrow{k_2} \mathrm{S} + \mathrm{D} \\ \mathrm{A} + \mathrm{S} \xrightarrow{k_3} 2\mathrm{D} \\ \mathrm{A} + \mathrm{D} \xrightarrow{k_4} 2\mathrm{D} \\ \mathrm{A} + \mathrm{D} \xrightarrow{k_5} \mathrm{S} + \mathrm{D} \\ \mathrm{A} + \mathrm{D} \xrightarrow{k_6} 2\mathrm{S} \\ \mathrm{S} \xrightarrow{d_1} \\ \mathrm{D} \xrightarrow{d_2} \end{array} \tag{1}$$

where k_i are rate constants and d_i are degradation rates. Cells only self-renew in the presence of nutrients and growth factors that are generically represented by A. It has been shown experimentally that, in *in vitro* cultures of breast cancer cells, all the transition described in (1) occur with positive rates, [5]. The degradation rates described by the two last diagrams in (1) will be used to simulate the inhibition effects of cytotoxic agents.

By the mass action law, [4], the concentrations of cells in culture evolve in time according to the system of differential equations,

$$\left\{ \begin{array}{rcl} \dot{S} & = & (k_1 - k_3)AS + (k_5 + 2k_6)AD - d_1 S \\ \dot{D} & = & (k_2 + 2k_3)AS + (k_4 - k_6)AD - d_2 D \\ \dot{A} & = & -(k_1 + k_2 + k_3)AS - (k_4 + k_5 + k_6)AD\,. \end{array} \right. \tag{2}$$

These equations describe the growth of a population of cells with two distinctive subpopulations in a culture medium. $S(t)$ and $D(t)$ represent the concentrations of stem-like and differentiated cells at time t, and $A(t)$ is the concentration of nutrients. This approach has been successfully used in cellular biology to describe quantitatively cell growth, [7], and provides the basic framework to describe qualitatively avascular tumour growth, [2].

Let us analyse first the dynamics of *in vitro* tumour growth. In this case, we have, $d_1 = d_2 = 0$.

With $d_1 = d_2 = 0$ in equation (2), we have $\dot{A} + \dot{S} + \dot{D} = 0$, obtaining the conservation law, $A(t) + S(t) + D(t) = A(0) + S(0) + D(0)$. From this conservation law, only two equations in (2) are independent, and the equations describing the concentration of the cells in culture over time simplifies to,

$$\begin{cases} \dot{S} &= ((k_1 - k_3)S + (k_5 + 2k_6)D)(c_0 - S - D) \\ \dot{D} &= ((k_2 + 2k_3)S + (k_4 - k_6)D)(c_0 - S - D) \end{cases} \tag{3}$$

where $c_0 = A(0) + S(0) + D(0)$ is a constant. Due to the dependence of c_0 on the initial conditions S_0 and D_0, the phase space of equation (3) is a right-angled triangle with one of the vertices at the origin of coordinates, figure 1. The length of the edges along the S and D directions is c_0. The hypotenuse of this triangle is a line of fixed points and is defined by the equation $(c_0 - S - D) = 0$. If $A(0) > 0$, any initial condition for equation (3) is represented in phase space as a point inside this triangle.

The system of equations (3) has an isolated fixed point at the origin of coordinates, $(S^*, D^*) = (0, 0)$. Then we have,

Proposition 1. *We consider the system of equations (3) with $c_0 > 0$ ($A_0 > 0$) and $k_i > 0$, for $i = 1, \ldots, 6$. Then, the zero fixed point of the system of equations (3) is Lyapunov unstable. Moreover, for any initial condition inside the triangular region delimited by the line $D = c_0 - S$ and depleted from the point $(0, 0)$, in the limit $t \to \infty$, the solutions of the system of equations (3) converge to points on the line $D = c_0 - S$.*

The proof of Proposition 1 is in Appendix A.

In figure 1, we show the qualitative structure of the phase curves of the system of equations (3).

As, for large values of t, we have approximately $D(t) = c_0 - S(t)$, we conclude that for large values of t, $D(t)/c_0 + S(t)/c_0 = 1$ and, asymptotically in time, the concentrations of the two subpopulations attain fixed proportions such that $D(t)/c_0 + S(t)/c_0 = D^*/c_0 + S^*/c_0 = 1$, where (S^*, D^*) is the coordinate on the line $D = c_0 - S$ of the limiting solution. It is not difficult to prove that that the zero fixed point of the system of equations (3) is a saddle point (see the proof of Proposition 1 in Appendix A).

Due to the conservation law $A(t) + S(t) + D(t) = A(0) + S(0) + D(0)$, asymptotically in time, the solutions of the system of equations (3) are bounded. If we had assumed a continuous supply of resources such that $A = constant$, the term $(c_0 - S - D)$ in (3) should be substituted by the constant A. In this case, it can be simply proved that, in the conditions of

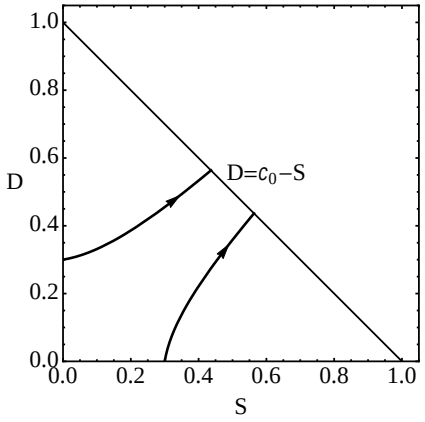

Figure 1: Phase space curves of the system of equations (3), for the parameter values, $k_1 = k_2 = k_4 = k_5 = 1$, $k_3 = k_6 = 0.5$, $A(0) = 0.7$, $c_0 = 1$, and two initial conditions $(S_0, D_0) = (0.3, 0)$ and $(S_0, D_0) = (0, 0.3)$. All the solutions with initial conditions in the interior of the triangular region converge as $t \to \infty$ to the line of fixed points $D = c_0 - S$, and the zero fixed point is unstable.

Proposition 1, the zero fixed point of the modified equation (3) is Lyapunov unstable, and the asymptotic solutions of the modified equation diverge to infinity, maintaining the fixed proportions D^*/c_0 and S^*/c_0. It is not difficult to see that that the zero fixed point of the system of equations (3) is a saddle point and the point (S^*, D^*) is the intersection of that tangent unstable manifold of the fixed point at the origin with the line $D = c_0 - S$.

The model just described shows the same type of temporal behaviour as the experimental observations of [5] (figure 3). It shows that, for the same kinetic parameters, the fixed proportions of cell-state populations depends only on the initial concentration of the subpopulations in culture.

3 Chemotherapy effects

Introducing a cytotoxic agent B in the *in vitro* culture and assuming that its effect is to destroy cells, we can describe this process by the kinetic mechanisms,

$$\begin{array}{l} \mathrm{B} + \mathrm{S} \xrightarrow{r_1} \\ \mathrm{B} + \mathrm{D} \xrightarrow{r_2} \end{array} \tag{4}$$

Comparing the two mechanism in (4) with the last two mechanisms in (1), with the identification $d_i = r_i \mathrm{B}$, and assuming a constant concentration of

the cytotoxic agent B, the system of equations (2) describes the therapeutic effect of B during the growth of culture cells. We could also have assumed two specific cytotoxic agents B_1 and B_2, but the identification would be similar.

The system of equations (2) describes the effect of a chemotherapeutic drug in *in vitro* culture of cells. In laboratories testing conditions, the effect of a drug can be analysed with this simple modelling approach. A necessary condition for the successfulness of a treatment leading to the elimination of cancer cells is the possibility of choosing constants $d_1 > 0$ and $d_2 > 0$ such that the solutions of the model equations (2) converge to the zero steady state in the variables S and D.

In model equations (2), it is assumed that cells duplicate in the presence of nutrients and growth factors, generically represented by A. Assuming the worst possible situation, where growth factors and nutrients are freely available in the cell culture, we can consider that A is a constant along all the process. In this case, the model equations (2) reduce to,

$$\begin{cases} \dot{S} &= (k_1 - k_3)AS + (k_5 + 2k_6)AD - d_1 S \\ \dot{D} &= (k_2 + 2k_3)AS + (k_4 - k_6)AD - d_2 D \end{cases} \tag{5}$$

where $d_i = r_i \mathrm{B}$, for $i = 1, 2$.

Generically, the linear equation (5) has a unique fixed point for $(S, D) = (0, 0)$. If, $d_1 = d_2 = 0$ and in the conditions of Proposition 1, the zero fixed point is unstable (see the proof of Proposition 1). A chemotherapy can only be successful if there is a choice of the positive constants d_1 and d_2 such that the zero fixed point of the system of equation (5) is Lyapunov stable. This implies that, in the presence of resources and growth factors, the cytotoxic agent is able to induce cell death rates that completely inhibits the growth of cell cultures.

Proposition 2. *We consider the system of equations (5) with $A > 0$ and $k_i > 0$, for $i = 1, \ldots, 6$. The zero fixed point of the system of equations (5) is Lyapunov stable if and only if one of the following conditions is verified:*

1) $d_1 > 0$, $d_2 = 0$, $d_1 > Ak_1$ *and* $k_6 > k_4$.

2) $d_1 = 0$, $d_2 > 0$, $d_2 > Ak_4$ *and* $k_3 > k_1$.

3) $d_1 > (k_1 - k_3)A$, $d_2 > (k_4 - k_6)A$ *and* $d_2 > (Z - A^2 k_1 k_4 - A d_1(k_6 - k_4))/(d_1 + A(k_3 - k_1))$, *where* $Z = A^2(k_1 k_6 + k_3 k_4 + k_2 k_5 + 2k_3 k_5 + 2k_2 k_6 + 3k_3 k_6)$.

The proof of Proposition 2 is in Appendix A.

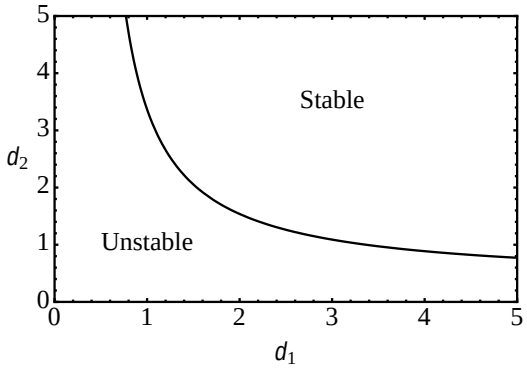

Figure 2: Stability region of the zero fixed point of the model equation (5), for the same parameter values of figure 1. The limit of the parameter region of stability of the zero fixed point is given in case 3) of Proposition 2. For this choice of parameter values and if d_1 and d_2 are sufficiently large, the cytotoxic agent has an inhibitory effect on the growth of cancer cells.

An immediate consequence of Proposition 2-3) is that, if $k_1 > k_3$ and $k_4 > k_6$, then $d_1 > (k_1 - k_3)A > 0$ and $d_2 > (k_4 - k_6)A > 0$. If the values of the degradation rates are sufficiently large, then the zero fixed point is Lyapunov stable, and there exists of an effective inhibitory effect of the cytotoxic agent over the cancer cells. In figure 2, we show the stability region of the zero fixed point of the model equation (5), for the same parameter values of figure 1. These situations can be achieved *in vitro* cell cultures by increasing the concentration of the cytotox agent ($d_i = r_i B$). But, *in vivo* cancer cells, screening effects due to the spatial extension of tumours must be taken into account.

If $k_1 < k_3$ and $k_4 < k_6$, the zero fixed point is Lyapunov stable for small values of d_1 and d_2.

If chemotherapeutic inhibitory effects target exclusively CSC, we have, by Proposition 2-1)-2), $d_2 = 0$ and if $k_4 > k_6$, the inhibitory effect of the cytotoxic agent over the cancer cells are inefficient for any value of $d_1 > 0$. Also, if $d_1 = 0$ and $k_1 > k_3$, cytotoxic agents will not eliminate cancer cells.

Thus, we have shown that in the presence of interconversion between non-stem and stem-like cancer cells and independently of the concentration of the cytotoxic agent, there are particular growth conditions where chemotherapy is not effective.

4 Discussion

We have derived a simple model to analyse the effectiveness of the cytotoxic therapies to eliminate cancer cells from *in vitro* cultures. We have shown that if the degradation rates induced simultaneously on cancer stem cell and on differentiated cancer cells are sufficiently large, then it is possible to eliminate cancer cells from a nutrient rich culture.

On the contrary, if cytotoxic agents target only cancer stem cells and if the rate of renewal of differentiated cells is larger than the rate of conversion of differentiated cells to cancer stem cells, then there is no effective strategy to eliminate cancer cells from *in vitro* cell cultures. For example, according to the quantitative results of Gupta *et al.*, [5], for the breast cancer cell culture SUM149, the probability of interconversion between basal (b) and steam-like (s) cells is $p_{b\to b} = 0.90 > p_{b\to s} = 0.01$ and, in this case, it is not possible to have an effective cytotoxic strategy targeting only CSC.

The chemotherapeutic elimination of *in vivo* cancer cell will be effective if it is effective in *in vitro* cultures, if it targets all the cancer cell types, and if the induced death rates for the different subpopulations are large enough.

Appendix A

Proof. Proposition 1. Inside the triangle $T = \{(S, D) \in \mathbb{R}^2 : S \geq 0, D \geq 0, D \leq c_0 - S, (S, D) \neq (0, 0)\}$, the integral curves of equation (3) coincide with the integral curves of the linear system,

$$\left\{ \begin{array}{rcl} \dot{S} & = & (k_1 - k_3)S + (k_5 + 2k_6)D \\ \dot{D} & = & (k_2 + 2k_3)S + (k_4 - k_6)D\,. \end{array} \right. \tag{6}$$

Therefore, assuming that the fixed point $(0, 0)$ of the system of equations (3) is Lyapunov unstable, the integral curves of equation (6) passing by $(S_0, D_0) \in T$ cross the line $D = c_0 - S$ in finite time, provided $c_0 > 0$. This proves the second part of the proposition.

To prove that the fixed point $(0, 0)$ of the system of equations (3) is Lyapunov unstable, we calculate the Jacobian matrix of (3) at $(0, 0)$, obtaining,

$$M = \begin{pmatrix} c_0(k_1 - k_3) & c_0(k_5 + 2k_6) \\ c_0(k_2 + 2k_3) & c_0(k_4 - k_6) \end{pmatrix} := \begin{pmatrix} c_0 x & c_0 c_1 \\ c_0 c_2 & c_0 y \end{pmatrix}.$$

Let us assume for the moment that the zero fixed point is Lyapunov stable with $c_0 > 0$ and $k_i > 0$, for $i = 1, \ldots, 6$. In this case, we have $\mathrm{Trace}M < 0$ and $\mathrm{Det}M > 0$. From these two conditions, with $c_0 > 0$, we obtain, $x + y < 0$

and $xy - c_1c_2 > 0$. So, if the fixed point is Lyapunov stable, we must have $x < 0$, $y < 0$ and $xy - c_1c_2 > 0$. But,

$$\mathrm{Det}M = k_4x - k_2k_5 - 2k_3k_5 - k_1k_6 - 2k_2k_6 - 3k_3k_6 < 0$$

which contradicts the initial assumption of stability of the fixed point $(0, 0)$. Therefore, $(0, 0)$ is unstable and the proposition is proved. □

Proof. Proposition 2. To prove that the fixed point $(0, 0)$ is Lyapunov stable, we calculate the Jacobian matrix of (5) at $(0, 0)$, obtaining,

$$M = \begin{pmatrix} A(k_1 - k_3) - d_1 & A(k_5 + 2k_6) \\ A(k_2 + 2k_3) & A(k_4 - k_6) - d_2 \end{pmatrix} := \begin{pmatrix} x & c_1 \\ c_2 & y \end{pmatrix}.$$

The fixed point is stable if $\mathrm{Trace}M < 0$ and $\mathrm{Det}M > 0$. From these two conditions, with $A > 0$, we obtain, $x + y < 0$ and $xy - c_1c_2 > 0$. Therefore, we must have $x < 0$, $y < 0$ and $xy - c_1c_2 > 0$. Let us write,

$$\mathrm{Det}M = d_1d_2 + d_1A(k_6 - k_4) + d_2A(k_3 - k_1) + k_1A^2(k_4 - k_6) - Z' \quad (7)$$

$$\mathrm{Det}M = d_1d_2 + d_1A(k_6 - k_4) + d_2A(k_3 - k_1) + k_4A^2(k_1 - k_3) - Z'' \quad (8)$$

$$\mathrm{Det}M = d_1d_2 + d_1A(k_6 - k_4) + d_2A(k_3 - k_1) + A^2k_1k_4 - Z \quad (9)$$

where,

$$\begin{aligned} Z' &= A^2(k_3k_4 + k_2k_5 + 2k_3k_5 + 2k_2k_6 + 3k_3k_6) > 0 \\ Z'' &= A^2(k_1k_6 + k_2k_5 + 2k_3k_5 + 2k_2k_6 + 3k_3k_6) > 0 \\ Z &= A^2(k_1k_6 + k_3k_4 + k_2k_5 + 2k_3k_5 + 2k_2k_6 + 3k_3k_6) > 0\,. \end{aligned}$$

If $d_1 > 0$ and $d_2 = 0$, by the trace conditions $x < 0$ and $y < 0$, we obtain, $A(k_1 - k_3) < d_1$ and $(k_6 - k_4) > 0$. By (7), $\mathrm{Det}M = d_1A(k_6 - k_4) + k_1A^2(k_4 - k_6) - Z' > 0$, which implies that, $A(d_1 - k_1A)(k_6 - k_4) > Z' > 0$. So we must have $A(d_1 - k_1A) > 0$ and $(k_6 - k_4) > 0$. Combining these conditions, 1) is proved.

If $d_1 = 0$ and $d_2 > 0$, by the trace conditions $x < 0$ and $y < 0$, we obtain, $k_1 < k_3$ and $A(k_4 - k_6) < d_2$. By (8), $\mathrm{Det}M = d_2A(k_3 - k_1) + k_4A^2(k_1 - k_3) - Z'' > 0$, which implies that, $A(d_2 - k_4A)(k_3 - k_1) > Z'' > 0$. So we must have $A(d_2 - k_4A) > 0$ and $(k_3 - k_1) > 0$. Combining these conditions, 2) is proved.

We consider now the case $d_1 > 0$ and $d_2 > 0$. By the trace conditions $x < 0$ and $y < 0$, we obtain, $A(k_1 - k_3) < d_1$ and $A(k_4 - k_6) < d_2$. By (9), we obtain,

$$d_2 > \frac{Z - A^2k_1k_4 - Ad_1(k_6 - k_4)}{d_1 + A(k_3 - k_1)}$$

and 3) is proved. □

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