Elucidating the Role Played by Cancer Stem Cells in Cancer Growth

Esclareciendo el papel que juegan las células madre cancerosas en el crecimiento del cáncer

Lucas Barberis[1,2], Lucia Benítez[1,2] and Carlos A. Condat[1,2]

[1] *Instituto de Física Enrique Gaviola, CONICET, Córdoba, Argentina*
[2] *FaMAF, Universidad Nacional de Córdoba, Córdoba, Argentina*

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Abstract— The cancer stem cell hypothesis states that cancer growth is propelled by a relatively small number of cancer stem cells (CSCs). These CSCs have been shown to play a crucial role in the growth and recurrence of many tumor types. The possibility that their elimination becomes an efficient cancer control procedure has even led to new therapeutic paradigms. On the other hand, from their early stages, most solid tumors grow in stressed environments. The stress field impacts on tumor evolution, and it is likely to affect different cancer cell populations in different ways. It is therefore of great interest to determine the nature and strength of the interactions between CSCs and differentiated tumor cells and how these interactions are affected by the mechanical properties of the environment. We have developed a two-population mathematical model suitable to describe the initial stages of cancer growth and applied it to extract information from three different experiments. Two of these experiments involve tumorspheres (spheroids resulting from the proliferation of a single CSC). In these cases, the model validates the concept of CSC niche (the microenvironment responsible for signals that stimulate or inhibit CSC growth), shows that interspecific interactions stimulate growth, while intraspecific interactions are generally inhibitory, and indicates how substrate hardness modifies growth. In the third experiment analyzed, where stress-induced growth suppression was measured in multicellular tumor spheroids, we were able to reconstruct the (unobserved) CSC fraction and found that medium rigidity eventually forces all cell interactions to be competitive. We find that, under adverse environmental conditions the CSC fraction always remains nonzero. This lends support to the hypothesis of the existence of the niche as a regulatory maintenance mechanism whose understanding will be crucial to the development of a successful therapy based on CSC elimination.

Keywords—cancer stem cell, tumor, populations, interaction modeling, substrate

Resumen— La hipótesis de las células madre cancerosas afirma que el crecimiento del cáncer es promovido por un número relativamente pequeño de células madre cancerosas (CSCs). Se ha demostrado que estas CSC juegan un papel crucial en el crecimiento y recurrencia de muchos tipos de tumores. La posibilidad de que su eliminación se transforme en un procedimiento eficiente para el control del cáncer ha conducido a nuevos paradigmas terapéuticos. Por otra parte, a partir de sus etapas iniciales la mayoría de los tumores sólidos crecen en ambientes sujetos a tensiones mecánicas. El campo de tensiones afecta a la evolución del tumor y es probable que afecte en forma diferente a las distintas poblaciones celulares. Es entonces de gran interés determinar la naturaleza e intensidad de las interacciones entre las CSC y las células cancerosas diferenciadas del tumor y cómo estas interacciones son influidas por las propiedades mecánicas del ambiente. Hemos desarrollado un modelo matemático de dos poblaciones, adecuado para describir las etapas iniciales del crecimiento del cáncer y lo hemos aplicado para obtener información a partir de tres experimentos diferentes. Dos de estos experimentos fueron realizados con tumoresferas (esferoides que resultan de la proliferación de una sola CSC). En estos casos, el modelo valida el concepto de nicho: el microambiente responsable por las señales que estimulan o inhiben la reproducción de las CSC, muestra que las interacciones interespecíficas estimulan el crecimiento, mientras que las interacciones intraespecíficas son generalmente inhibitorias, e indica cómo la rigidez del substrato modifica el crecimiento. En el tercer experimento que analizamos, en el que fue medido cómo la presión aplicada suprime el crecimiento de esferoides multicelulares, pudimos reconstruir la fracción de CSC (que no había sido medida) y encontramos que la rigidez del medio eventualmente hace que todas las interacciones intercelulares sean competitivas. Encontramos también que bajo condiciones ambientales adversas la fracción de CSC siempre es no nula. Estos resultados apoyan la hipótesis de la existencia del nicho como mecanismo de mantenimiento regulatorio cuya dilucidación es esencial para el desarrollo de una terapia exitosa basada en la eliminación de las CSC.

Palabras clave—célula madre cancerosa, tumor, poblaciones, modelado de interacciones, substrato

INTRODUCTION

The obvious interest and complexity of cancer has led to the formulation of numerous mathematical models of tumor growth. Surveys of the state of the art in cancer modeling have appeared at different times (Adam and Bellomo, 1997; Wodarz and Komarova, 2005; Tan and Hanin, 2008; Lowengrub et al., 2009). The models range from the deterministic differential equation descriptions (Murray, 2001) to the cellular automata (Scalerandi et al., 1999), to stochastic approaches (Tan and Hanin, 2008). The emergence of new experimental techniques continuously imposes further constraints on the growth models being developed. Multicellular tumor spheroids were developed to study the influence of the microenvironment on the regulation of cell development and viability (Freyer and Sutherland, 1986; Sutherland, 1988). Later, the identification of cancer stem cells in many tumor types (Al-Hajj et al., 2003; O'Brien et al., 2007) led to the formulation of the cancer stem cell hypothesis (Batlle and Clevers, 2017), which states that a subpopulation of stem cells drives cancer growth and may explain metastasis and tumor recurrence after therapy. As a consequence, new therapeutic paradigms have emerged, built on the idea of controlling cancer through the destruction or incapacitation of the CSCs (Jagust et al., 2019). Tumorspheres were developed as biological models to test the potential and weaknesses of CSC – driven tumor growth (Weiswald et al., 2015). They are spheroids grown from single-cell suspensions out of permanent cell lines or tumor tissue. When cultivated in a serum-free medium, they grow in a natural way, but their environment can be tuned-up to study particular phenomenologies. By adding growth factors such as EGF and FGF-2, the stem cells are forced to self-replicate without differentiating. Another possibility is to grow the spheroids in agarose gel to induce stress and examine how this stress modifies cell behavior and proliferation.

Associated with a stem cell is the *niche*, the set of cells and intercellular elements that provide the signals that define stem cell behavior and maintain stemness (Scadden, 2014). In the case of CSCs, the niche functionality is well known, but its design and structure are still not precisely defined, although its deregulation is certainly connected to the emergence of tumorigenesis. The cross-talk between the CSCs and their niches has now become a possible therapeutic target (Taniguchi et al., 2020). Mechanical stresses are important to the initiation and interpretation of the promoting and inhibitory signals that the niche sends to the CSC (Cheng et al., 2009). To shed light on the niche properties and on the effect of mechanical interactions, it is therefore important to have a mathematical model to describe stem-cell-fueled tumorsphere growth. We stress the importance of having a simple mathematical model: Tumorsphere experiments are notoriously difficult, and they yield key but relatively scarce data. Such sophisticated models as the one developed in (Scalerandi et al., 2002) to explain the growth of tumor cords under varying stresses are not useful to extract information of the available tumorsphere data.

We have recently developed a two-population mathematical model of tumor growth (Benítez et al., 2019, 2021), which we used to interpret the results of tumorsphere growth experiments performed under different mechanical and growth factor conditions and to extract information about the interaction between cancer stem cells and differentiated cancer cells. We also obtain the evolution of the CSC fraction, an important quantity which is in general not directly accessible to the experiments. Here we review the model, present its properties and applications, and indicate how to apply it to interpret the results of tumorsphere and spheroid growth experiments.

THE MATHEMATICAL MODEL

When a CSC undergoes mitosis, it may generate either two CSCs with a probability p_s, two differentiated cancer cells (DCCs) with a probability p_d, or one CSC and one differentiated CSC, with a probability $p_a = 1 - p_s - p_d$. To describe the interaction between the CSC and DCC populations we must generalize the standard equations for competing species to account for these three possible results of a CSC division. Since it is in general not possible to discriminate between the growth rates of the two populations, we will assume that all cells divide at the same basal reproductive rate r. The interactions between members of both populations will be quantified by four coefficients α_{ij} , which describe intraspecific ($i = j$) and interspecific ($i \neq j$) interactions.

Let $S(t)$ and $D(t)$ be, respectively, the CSC and DCC populations at time t. Their evolution is then given by the following pair of equations:

$$\frac{dS}{dt} = r[p_s S]\left\{\frac{p_s - p_d}{p_s} - \alpha_{SS}S - \alpha_{SD}D\right\}, \tag{1a}$$

$$\frac{dD}{dt} = r[D + (1 + p_d - p_s)S]\left\{1 - \alpha_{DD}D - \alpha_{DS}S\right\}. \tag{1b}$$

Positive (negative) values of the coefficient α_{ij} describe the inhibition (promotion) of population i growth by the presence of population j, i.e. positive values of α_{ij} correspond to competitive interactions, while negative values represent cooperation. The initial conditions for these equations depend on the system of interest. In the case of a tumorsphere, we start with a seed of a single cancer stem cell given by $S(0) = 1$ and $D(0) = 0$, but other choices are possible.

Since our system of equations is designed to describe cell populations, we should determine a positivity conditions for its solutions. A sufficient condition may be obtained following the method of Kirwa *et al.* (see Chen et al. (2016)), and noting that in all systems of interest $S(0) > 0$ and $D(0) \geq 0$. We thus have,

Theorem 1 *Let $S(t)$ and $D(t)$ be continuous functions that solve Eqs.* (1)*, and satisfy $S(0) > 0$ and $D(0) = 0$. Then $\alpha_{DS} \leq 0$ is a sufficient condition for the positivity of $S(t)$ and $D(t)$.*

Proof: Given that $S(0) > 0$, for $S(t)$ to become non-positive for the first time at a $t_0 > 0$, we must require that $S(t_0) = 0$ and $S'(t_0) < 0$. But replacing these in Eq. (1a), we arrive at a contradiction: while the left-hand side is negative definite, the right-hand side is zero. Therefore, $S(t)$ must be positive for any finite times, without any conditions on the model parameters.

To obtain the positivity condition for $D(t)$, we note that a sufficient condition for the initial growth of $D(t)$ is $\alpha_{DS} \leq 0$.

In this case, there must be at least an interval $(0,t_1)$ where $D(t) > 0$. If we now assume that t_1 is the first positive time for which D vanishes, $D(t_1) = 0$, then $D'(t_1) < 0$. Since $r > 0$ and $S(t_1) > 0$, Eq. (1b) requires that $[1 - \alpha_{DS} S(t_1)] < 0$, a condition that is never fulfilled because $\alpha_{DS} \leq 0$. Thus, we have again arrived at a contradiction and $D(t)$ must remain positive for all finite times.

The generalization of this theorem to the case $D(0) > 0$ is straightforward and we will not go into the details here.

The condition $\alpha_{DS} \leq 0$ is likely to be generally fulfilled because we expect the cancer stem cells to promote the growth of the differentiated cells that make up their niche.

Initial size

A natural question for a biologist would be: If I want to create a tumorsphere, what is the minimum size that should be used to ensure growth? The answer to this question depends on the influence of differentiation inhibitors, as we can see by requiring that the time derivative $S'(t)$ of the CMC number be positive at the start of the experiment. From Eq. (1a), we see that this is equivalent to demanding that the initial seed size satisfy the inequality,

$$1 - \Pi - \alpha_{SS} S(0) > 0, \tag{2}$$

where $\Pi = p_d/p_s$. If $\alpha_{SS} < 0$ (the CMCs cooperate), this is equivalent to,

$$S(0) > \frac{\Pi - 1}{|\alpha_{SS}|}. \tag{3}$$

If $\Pi < 1$, i.e. $p_d < p_s$, any value of $S(0)$ (for instance, a single cancer stem cell) will suffice to start a tumorsphere. If on the other hand, $\Pi > 1$, Eq. (3) provides us with a convenient estimate for the seed size.

If $\alpha_{SS} > 0$, the CMCs inhibit each other, and Eq. (3) is replaced by

$$S(0) < \frac{1 - \Pi}{\alpha_{SS}}. \tag{4}$$

Because of the intraspecific competition, even if $p_s > p_d$, the number of cancer stem cells will initially decrease unless Eq. (4) is satisfied.

To further investigate the mathematical properties of the System (1), it is convenient to write it in a dimensionless form. To do this, we will assume that $\alpha_{DD} \neq 0$. If we then define the dimensionless time $\tau = rt$ and the dimensionless populations,

$$X = \alpha_{DD}(1 - p_s + p_d) S, \tag{5a}$$

$$Y = \alpha_{DD} D, \tag{5b}$$

we obtain a set of dimensionless equations:

$$\dot{X} = (P - AX - BY) X, \tag{6a}$$

$$\dot{Y} = (1 - CX - Y)(X + Y), \tag{6b}$$

where the dot signals a derivative with respect to τ and we have defined the new parameters

$$P = p_s - p_d, \tag{7a}$$

$$A = \frac{\alpha_{SS}}{\alpha_{DD}} \frac{p_s}{1 - p_s + p_d}, \tag{7b}$$

$$B = \frac{\alpha_{SD}}{\alpha_{DD}} p_s, \tag{7c}$$

and

$$C = \frac{\alpha_{DS}}{\alpha_{DD}} \frac{1}{1 - p_s + p_d}. \tag{7d}$$

We have already shown that the sufficient condition for the positivity of the solutions to Eqs. (1) is that $\alpha_{DS} \leq 0$. From Eqs.(6) we see that X and Y will have the same (opposite) signs than their dimensional counterparts if $\alpha_{DD} > 0$ (< 0). Using Theorem 1 and Eq. (7d), we arrive at the following corollary:

Corollary 1 *The simultaneous satisfaction of the conditions $\alpha_{DS} \leq 0$ and $\alpha_{DD} > 0$ implies the positivity of $X(\tau)$ and $Y(\tau)$. The single condition $C \leq 0$ may be used instead.*

Remark 1 *In most cases, we expect cancer stem cells to compete for space and resources, which would lead to $\alpha_{DD} > 0$. If they cooperate instead, $\alpha_{DD} < 0$ and the positivity of $S(t)$ and $D(t)$ would imply the* negativity *of $X(\tau)$ and $Y(\tau)$.*

Short-time behavior

Next, we investigate the short time behavior of system (6). This is given by,

Theorem 2 *If the system evolution starts at $\tau = 0$ from a small mixed seed, such that $X(0) = X_0 > 0$ and $Y(0) = Y_0$ but AX_0, CX_0, BY_0, and Y_0 are all much smaller than one, the representative point in the phase portrait of Eqs.* (6) *initially moves upwards and to the right. If $X_0 = 0$ but $Y_0 > 0$, the representative point moves straight towards the point* $(0,1)$.

Proof: Let us consider first the case $X_0 > 0$. Under the theorem assumptions, Eqs. (6) may be linearized. The explicit solutions to the linearized form are easy to obtain. They are

$$X(\tau) = X_0 \exp(P\tau), \tag{8a}$$

and

$$Y(\tau) = \left(Y_0 + \frac{X_0}{1 - P} \right) e^{\tau} - \frac{X_0}{1 - P} e^{P\tau}. \tag{8b}$$

From these equations, we immediately find that the short-time location of the representative point in the $X - Y$ plane is given by the equation

$$Y(t) = \left[Y_0 + \frac{X_0}{1 - P} \right] \left[\frac{X(t)}{X_0} \right]^{\frac{1}{P}} - \frac{X(t)}{1 - P}. \tag{9}$$

Given that $0 \leq P \leq 1$ and that $X(\tau) > X(0)$ for all $\tau > 0$, the trajectory will be initially controlled by the first term of Eq. (9), starting as a curve that moves upwards and to the right, as we wanted to prove. If $X_0 = 0$, Eq. (6a) leads to $X(\tau) = 0$, $\forall\, \tau > 0$. Equation (6b) is therefore elementary to integrate, yielding

$$Y(\tau) = \left[1 + \left(Y_0^{-1} - 1 \right) \exp(-\tau) \right]^{-1}, \tag{10}$$

an expression that is valid $\forall \tau > 0$. Equation (10) tells us that $Y(\tau \to \infty) = 1$ for any value of Y_0.

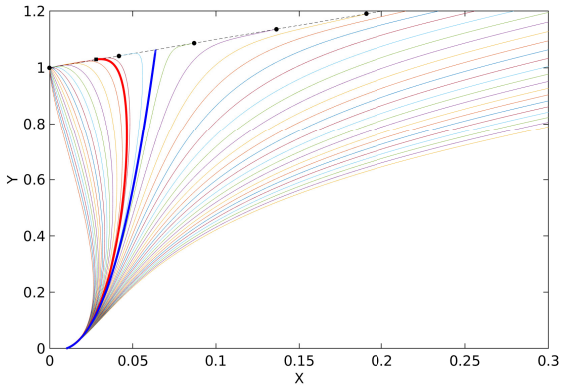

Figure 1: System evolution in the $X-Y$ plane for growth starting from a pure CSC seed when a mixed asymptotic population is reachable. Trajectories are obtained by varying the parameter B. Fixed parameter values: $P = 0.5$, $A = 2$, and $C = 0.1$. The red line corresponds to the bifurcation condition $B = P$. Lines to its left correspond to $B > P$ and end at the stable coexistence fixed point $Q_1 = (0,1)$. Lines to the right are obtained for $B < P$ and end at the stable coexistence point. The blue line corresponds to $B = \tilde{B}$ (see text).

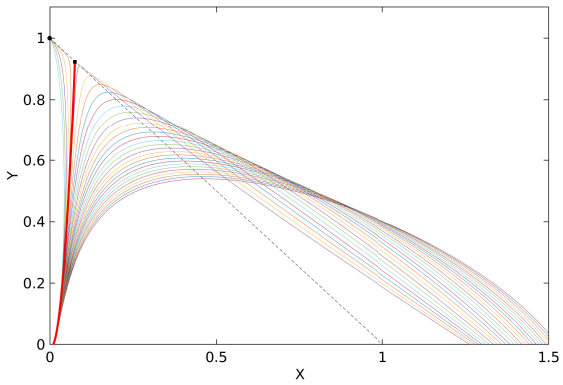

Figure 2: System evolution in the $X-Y$ plane for growth starting from a pure CSC seed, when a mixed asymptotic population is not reachable. Trajectories are obtained by varying the parameter B. Fixed parameter values: $P = 0.5$, $A = 0.1$, and $C = 1$. The red line corresponds to the bifurcation condition $B = P$. Lines to its left ($B > P$) end at the stable fixed point at $(0,1)$, while lines to its right ($B < P$) end at the X axis. Q_2 is never a stable fixed point. The fixed points, located along the dashed line, are not stable.

Fixed points and stability

The behavior of a cell colony can be studied by locating the equilibria (fixed points) of system (6) and analyzing their stability. The fixed points are determined by setting the right-hand sides of Eqs. (6) equal to zero. Their stability is then found by looking at the real parts of the eigenvalues λ_1 and λ_2 of the Jacobian matrix,

$$J = \begin{pmatrix} P - 2AX - By & -BX \\ 1 - 2CX - (1+C)Y & 1 - (1+C)X - 2Y \end{pmatrix} \qquad (11)$$

The fixed points Q_j are:

- The trivial point $Q_0 = (0,0)$, for which the eigenvalues are $\lambda_1 = 1$ and $\lambda_2 = P$. This point is unstable if $P > 0$, which is the most interesting case (cancer stem cells are stimulated to divide symmetrically), and the colony will grow. If $P < 0$, the origin is a saddle point.

- The differentiated cancer cell point, $Q_1 = (0,1)$, whose eigenvalues are $\lambda_1 = -1$ and $\lambda_2 = P - B$. This corresponds to a stable equilibrium if $P < B$ and is otherwise a saddle point.

- The coexistence point,

$$Q_2 = (X^*, Y^*) = \frac{1}{\Delta}(P - B, A - PC), \qquad (12)$$

  with $\Delta = A - BC$. The corresponding eigenvalues are too cumbersome for a direct interpretation, but extensive simulations confirm that Q_2 is unstable when $P < B$, which is the domain where Q_1 is stable. There is therefore a transcritical bifurcation at $P = B$, where an exchange of stability occurs: a stable equilibrium such that the system contains no cancer stem cells is found if $P < B$, while a stable equilibrium where both populations coexist occurs for $P > B$.

- The non-biological point $Q_3 = \frac{P}{A-B}(1,-1)$, for which one of the two populations would be negative.

The fixed points in the $X-Y$ plane are located on the straight line $Y^* = 1 - CX^*$. Since we generally expect $\alpha_{DD} > 0$ (differentiated cancer cells compete for resources), the sign of the slope of the fixed-point line is determined by the sign of α_{DS} (see Eq. (7d). If CSCs promote the generation of new differentiated cells ($\alpha_{DS} < 0$), an increase in the number of CSCs in equilibrium would imply an increase of the number of differentiated cells. Conversely, if the CSCs inhibit the creation of new differentiated cells ($\alpha_{DS} > 0$), an increase in the number of CSCs in the equilibrium would imply fewer DCCs there.

Figure 1 depicts the system trajectories in the $X-Y$ plane starting from a small CSC seed, for the parameter values indicated in the figure caption. After an initial stage where both populations increase in agreement with Theorem 2, we can identify three types of behavior: For $B > P$, all trajectories converge to the differentiated cancer cell fixed point Q_1, which represents a stable tumorsphere that contains no cancer stem cells. The transcritical bifurcation occurs at $B = P$, where there is an exchange of stability between Q_1 and Q_2, the last becoming the stable fixed point for $B < P$. If $P > B > \tilde{B}$, where $\tilde{B}$ is determined numerically, the populations go through a maximum, and then converge to a B-dependent coexistence point. If $B < \tilde{B}$, the tumorsphere grows monotonically towards the coexistence fixed point. Since $C < 0$, the slope of the fixed-point line is positive.

If $\Delta < 0$, but $B > P$ and $A < PC$, the coexistence fixed point is a saddle point located along the dashed line in the first quadrant. This case is shown in Fig. 2, where a typical value of Q_2 is represented by a square symbol. The trajectories cross into the fourth quadrant, but biological evolution ends on the X axis, which corresponds to a spheroid with no differentiated cancer cells.

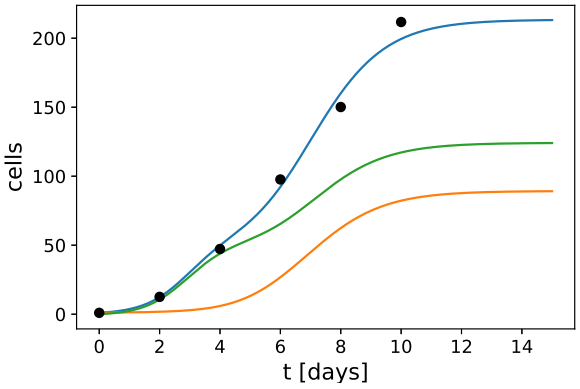

Figure 3: Fit to the experimental data (dots) of (Chen et al., 2016) to reconstruct the CSC population, which is given by the orange line. The green line corresponds to the DCC population, and the blue line is the fit to the total cell population.

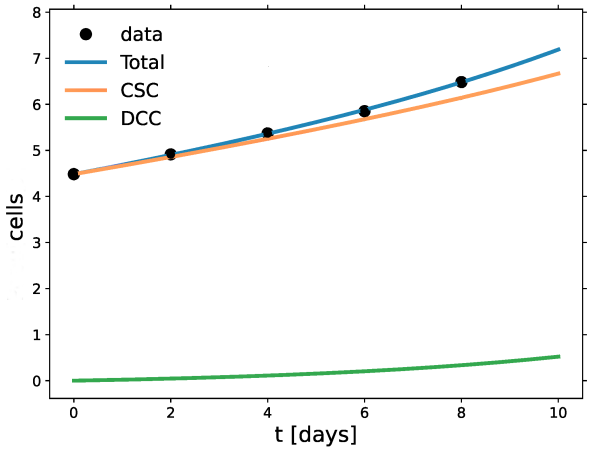

Figure 4: Fit to the experimental data (dots) of (Wang et al., 2016) to study tumorsphere growth on a soft substrate. The CSC population (red line) is much larger than the DCC population (green line).

THE MATHEMATICAL MODEL IN THE REAL WORLD

As stated in the Introduction, the model can describe the possible outcomes of a spheroid growth assay. To illustrate the application of our results to experimental data, we selected three experiments designed to investigate very different properties of a growing spheroid. The fitting parameters we obtain are reported in Table 1 along with the predicted final cell population and the expected CSC fraction. Note that the spheroids final sizes, in the column labeled "Total", span five orders of magnitude. We briefly discuss our interpretation of these results bearing in mind that our main goal is to predict the dynamics of the cancer stem cell fraction.

The first experiment we analyze corresponds to tumorsphere assays performed by Chen et al. (2016) starting from three cancer cell lines. They found that, under suitable growing conditions, the final CSC fraction is rather large. Here we report only results for the T47D breast cancer line, but a more detailed account may be found in Benítez et al. (2019). As seen in Fig. 3, the fitting agrees well with the data, and allows us to reconstruct the evolution of the CSC population. Growth stops after about fifteen days, as is usually observed, leaving the final CSC fraction stable. By inspection of the corresponding parameters in Table 1, we conclude that growth is favored by the interspecific interactions. Stem cells promote the increase in the number of differentiated cells that consolidate their niche, which leads to an initial decrease in the CSC fraction (see Fig. 6). Although the location of the cell population in the tumorsphere was not studied in the experiment, it has been recently suggested (Barberis, 2021) that the relatively high differentiation probability in combination with the colony's geometry helps the CSCs to build a DCC shield around them. As expected, the coefficients α_{DD} and α_{SS} are positive, confirming that cells in the same population compete for resources.

The second experiment Wang et al. (2016) studied the influence of the substrate on tumorsphere growth in CSC-promoting media. Recently, we applied our model to this experiment (Benítez et al., 2021). Here we report only the case of a "soft" substrate, in which cells were cultured using 0.05 % agar as the contact matrix surface. Growth is slow at first, being apparently driven only by the tendency to build a suitable niche, hence the small basal growth rate (c.f. Table 1). As a result, a slow exponential growth of CSCs prevails in the early stages. As in the preceding example, the CSCs attempt to generate niche-building DCCs, but, due to the strong differentiation inhibition forced by the addition of differentiation-inhibiting agents (note the high value of p_s), the CSCs are only occasionally able to generate a differentiated cell. Thus, as shown in Fig. 4, the CSC fraction is close to one with only a very slow decay. The signs of the coefficients α_{ij} again indicate interspecific cooperation and intraspecific competition.

The third experiment is a classic (Helmlinger et al., 1997). These researchers prepared culture media that induced increasing stresses on the growing tumor spheroids, showing that stress may be a strong growth inhibitor for tumors. Although these experiments were satisfactorily described using an allometry-based mathematical model (Delsanto et al., 2004), no information was extracted about subpopulations or the nature of the intercellular interactions. Now we fitted their reported tumor sizes with our model and found an interesting fact: the fit is not possible without the assumption of the existence of at least one CSC to drive the tumor growth. Here we report only our fit to the 0.3 % agarose concentration case, which corresponds to a relatively low stress environment. As shown in Fig. 5, the DCCs form an overwhelming majority of the cell population. We note that this experiment was performed at a time when the cancer stem cells were little more than a conjecture. The finding that those experiments must have been CSC-driven becomes evident today, because the currently accepted biological definition of a CSC states that it is a cell that can form a sphere in a tumorsphere assay (the original paper indicates that many cells were seeded but not all of them formed spheres). The CSC fraction represented in Fig. 6 for this experiment (green curve) shows that the pressure quickly kills the seed. As a result, the final size of the spheroid is reduced respect to the control one (not shown here), for which the CSC num-

TABLE 1: PARAMETERS OBTAINED BY FITTING THE DATA FROM THREE VERY DIFFERENT TUMORPHERE ASSAYS.

	α_{SS}	α_{SD}	α_{DS}	α_{DD}	r	p_s	p_d	Total	$\frac{S}{S+D}$
Chen	0.0519	-0.032816	-0.0175	0.020616	1.32	0.36	0.160	210	0.42
Wang	0.0844	-0.4082	-0.2005	0.483	0.07	0.969	0.004	7	0.92
Helmlinger	0.0202	0.000060	0.4007	0.000066	0.89	0.24	0.22	15178	0.00

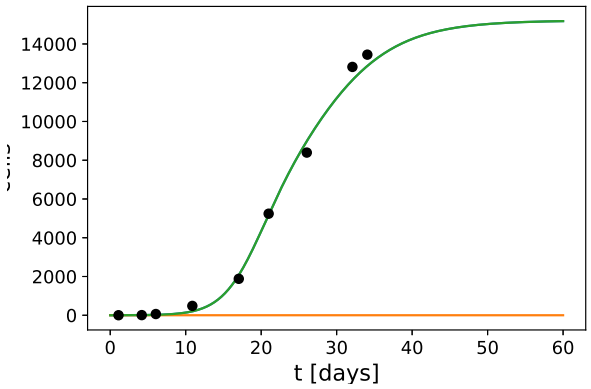

Figure 5: Fit (green line) the experimental data (dots) of (Helmlinger et al., 1917) for the total number of cells yields the evolution of the CSC population (orange line). Cancer stem cells cannot thrive due to the external pressure. Spheroids were grown in 0.3 % agarose gels.

ber becomes constant after a brief transient. The last result is consistent with the "control" experiment of Wang et al. (2016), analyzed by us in Benítez et al. (2021), and with the simulations reported in Barberis (2021).

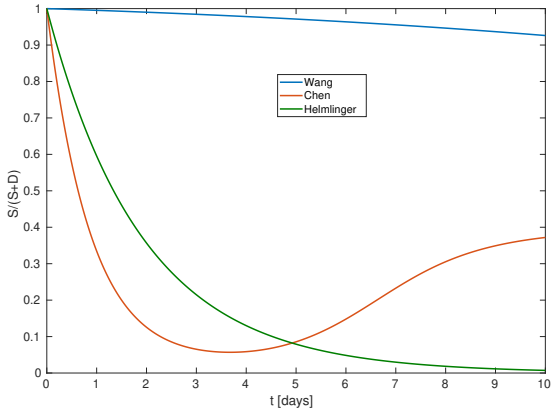

Figure 6: Time evolution of the CSCs fractions generated by our model for the experiments described in Figs. 3 to 5. Environmental factors alter the CSC fraction drastically.

CONCLUSION

The mathematical properties of a model for tumor spheroid growth driven by cancer stem cells were discussed in detail and illustrated through representative phase portraits. The model was then shown to be able not only to reproduce the extant data for tumorsphere growth (which usually correspond to whole populations), but to yield in each case the cancer stem cell fraction, which had not been accessible to the experimentalists.

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