XI CONGRESO LATINOAMERICANO
DE BIOLOGÍA MATEMÁTICA
SoLaBiMa 2019
Universidad Católica del Maule
22-25 de Octubre de 2019, Talca, Chile

Do cancer stem cells affect tumor spheroid growth under mechanical stress?

Lucía Benítez, Lucas Barberis, Carlos A. Condat*
Instituto de Física Enrique Gaviola, CONICET
Facultad de Matemática, Astronomía, Física y Computación,
Universidad Nacional de Córdoba.
Ciudad Universitaria, CP:X5000HUA Córdoba, Argentina

Abstract

Mechanical resistance of the surrounding tissue impacts on solid tumor progression. Tumor spheroids are experimental models for cancer growth which are also used to investigate the effect of stress on cancer dynamics. Here we show that a two-population model recently developed to describe the influence of cancer stem cells on tumorsphere growth can be applied to assess how the behavior of stem and differentiated cancer cells is affected by stresses. Comparison with experimental data suggests that stress makes the competition between differentiated cells stronger and weakens the competition among cancer stem cells, which improves tumor viability.

Introduction

Increasing experimental evidence is leading to the generalized acceptance of the cancer stem cell hypothesis, which postulates that, in many tumors, a subpopulation of the cancer cells, the cancer stem cells (CSCs), have the ability for self-renewal and can initiate and drive cancer growth [1]. Given the resistance of CSCs to therapy, incapacitating them could lead to cancer containment. It is therefore critical to elucidate their role in tumor dynamics. On the other hand, the mechanical properties of the tumor environment are determinant for its progression. Since stresses can affect growth and metastasis, there are numerous studies that use spheroids to evaluate tumor growth under mechanical stress [2-4]. The question we want to address here is: What are the implications

*Partially supported by SECyT-UNC (Project No. 05-B457) and CONICET (PIP 11220150100644), Argentina.
e-mail: condat@famaf.unc.edu.ar

of the presence of CSCs on a tumor subject to pressure?

We have recently developed a two-population mathematical model to describe CSC-driven tumorsphere growth [5]. This model was successfully applied to describe experimental results [6] for tumorspheres grown from three breast cancer cell lines. This application revealed that, while interspercific interactions stimulate growth, intraspecific interactions are inhibitory. The model exhibits three fixed points: an unstable point where the cancer colony disappears, a differentiated cell fixed point where all CSCs disappear, but a finite population of differentiated cancer cells remains, and a coexistence fixed point where finite numbers of differentiated cells and CSCs co-occur. By moving the relevant parameters, we found that there is a tipping point, characterized by a transcritical bifurcation, where the differentiated cancer cell attractor is replaced by the coexistence attractor. By applying the model to experimental data sets of spheroids grown under stress conditions, we will draw conclusions about the effect of pressure on cell-cell interactions.

The Model

Our model describes the evolution of the two interacting populations, CSCs, whose number is $S(t)$, and differentiated cancer cells, whose number is $D(t)$, by the following equations [5],

$$\frac{dS(t)}{dt} = r\,(p_s - p_d)S(t) - r\,p_s S(t)\Big(\alpha_{SS}S(t) + \alpha_{SD}D(t)\Big) \tag{1}$$

$$\frac{dD(t)}{dt} = r\,\Big[D(t) + [1-(p_s-p_d)]S(t)\Big]\Big[1 - (\alpha_{DD}D(t) + \alpha_{DS}S(t))\Big] \tag{2}$$

This version of the model is slightly different from that presented in Ref. [5]. Here, in the absence of more precise information, both subpopulations are assumed to have the same intrinsic division rate r. When an S cell divides, there is a probability p_s that two new S cells are generated and a probability p_d that two D cells are generated; the probability that there is an asymmetric division is $p_a = 1 - p_d - p_s$ (asymmetric divisions do not change the number of CSCs). The diagonal elements of the matrix α_{ij} describe intraspecific interactions, while its non-diagonal elements describe interspecific interactions. With this notation, $\alpha_{ij} < 0$ ($\alpha_{ij} > 0$) corresponds to *cooperative* (*inhibitory*) interactions. Plasticity, which is regulated by the microenvironment, is also expressed through these interaction coefficients.

The stability condition for the differentiated cancer cell attractor is,

$$\frac{\alpha_{SD}}{\alpha_{DD}} > 1 - \Pi \tag{3}$$

with $\Pi = \frac{p_d}{p_s} \leq 1$. If the system parameters satisfy condition (3), the resulting tumor is composed of $D_1 = \frac{1}{\alpha_{DD}}$ differentiated cancer cells. Otherwise the coexistence fixed point is stable, the corresponding cell numbers being,

$$S^* = \frac{\alpha_{DD}(1-\Pi) - \alpha_{SD}}{\delta} \tag{4}$$

$$D^* = \frac{\alpha_{SS} - (1-\Pi)\alpha_{DS}}{\delta} \tag{5}$$

Here $\delta = \alpha_{DD}\alpha_{SS} - \alpha_{SD}\alpha_{DS}$.

Comparison to experiments

Helmlinger and coworkers performed an extensive series of experiments to determine the effects of stress on the growth of LS174T multicellular spheroids [2]. Some of their results are presented as blue squares in the figures (we have replaced the spheroid diameters by cell numbers in order to facilitate comparisons). Figures 1 and 3 show the results for two sets experiments (I and II) where the sizes of free growing spheroids were measured. These results have been fitted with our model. In all the figures green lines represent the best fits of S + D to the total cell populations, while red lines correspond to our results for the stem cell populations, which were not measured in the experiments. The fitting parameters are presented in the Table. The fitting parameters corresponding to both free growth experiments are consistent and indicate that interspecific interactions are cooperative (α_{DS} and α_{SD} are negative), while the intraspecific interactions are inhibitory, revealing the presence of competition (α_{SS} and α_{DD} are positive). This is in agreement with what we found from fitting our model to the results of tumorsphere experiments where growth was started from single stem cells of the SUM 159, MCF-7, and T47D cancer cell lines [5]. In all figures the predicted numbers of CSCs are small and we have multiplied them by 1000 to represent them in the same graph as the differentiated cells.

Figure 2 shows the results for spheroids grown in a 0.8% agarose gel; the corresponding parameters are presented in the Table and suggest that, as a consequence of pressure (a) the competitive interactions among CSCs have weakened (α_{SS} is much smaller), while CSCs now inhibit the growth of differentiated cells (α_{DS} is positive), and (b) differentiated cancer cells now inhibit CSC population growth (α_{SD} is also positive) but the strength of the competitive interaction among differentiated cancer cells (α_{DD}) is roughly preserved. No reasonable fits could be obtained if there were no cancer stem cells present.

The same conclusions may be drawn from our fittings to the results of experiment II (See Fig. 4 and Table). In this experiment, the pressure generated by the agarose was released on day 30, and the spheroids were left to grow freely again. The first 30 days are very well described by the parameters presented in the last row of the Table. After day 30 we plot a curve constructed using the parameters in the third row of the Table with the value obtained for day 30 from the fit to the first 30 days as a starting point. We see that we then get a good agreement for the last three experimental points. To generate the fit we neglected the experimental point corresponding to $t = 22$ days, because it seems to be a clear outlier. We remark that at least one stem cell must be present at day 30 to allow for the observed fast resumption of growth.

Table. Fitting parameters for the experiments in Ref. [2].

	α_{ss}	α_{sd}	α_{ds}	α_{dd}	r
Free suspension I	0.8756	-0.000119	-0.5645	0.000089	0.7663
0.8 % agarose gel I	0.1094	0.000032	0.4206	0.000099	0.9381
Free suspension II	0.6608	-0.000095	-0.4302	0.000074	0.6923
0.8 % agarose gel II	0.2369	0.000097	0.5831	0.000139	1.0930

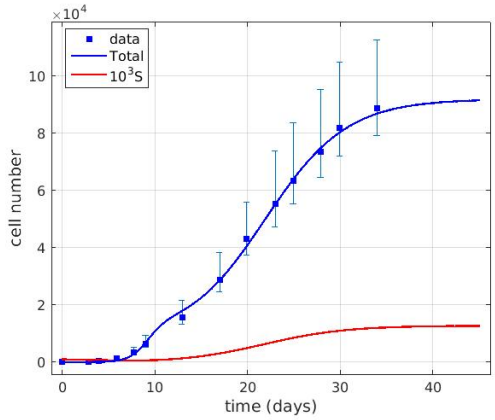

Figure 1: Free suspension I

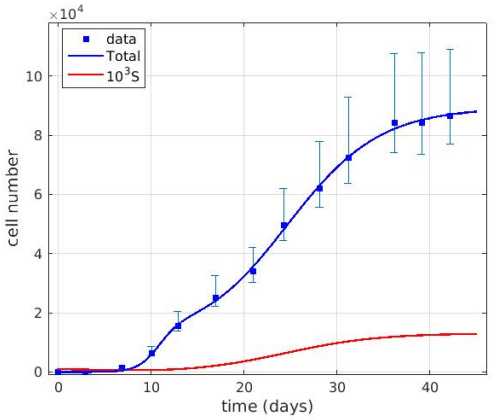

Figure 2: 0.8 % agarose gel I

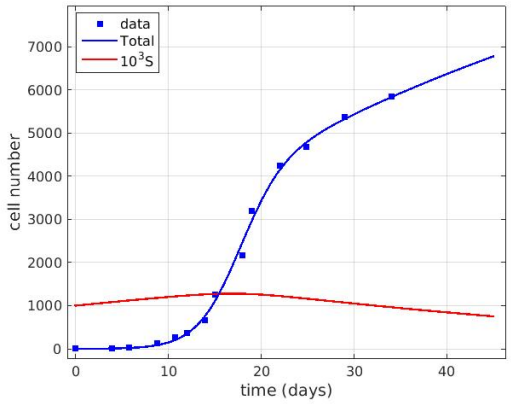

Figure 3: Free suspension II

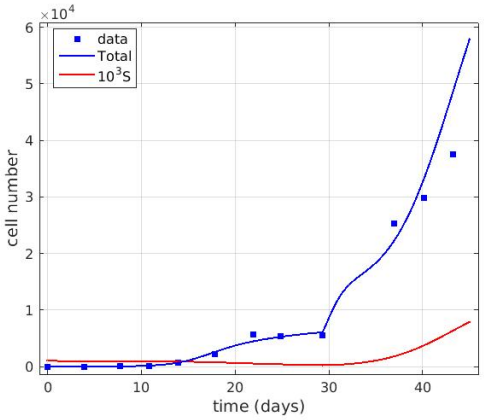

Figure 4: 0.8 % agarose gel II

Discussion

We conclude that:

1) The model gives a good description of spheroid growth and points to the existence of cancer stem cells in the experiments of Ref. [2]. No acceptable fits were obtained using a single cell population.

2) Since the spheroids in Ref. [2] were not specifically prepared to observe cancer stem cell development, it is not surprising that the fraction of cancer stem cells is lower than in Ref. [6].

3) Stress makes the competition between differentiated cells stronger, but the competition among cancer stem cells weakens, which improves tumor viability.

4) Stress increases the cell division rate.

5) A condition for tumor growth recovery after stress release is the survival of at least one stem cell.

References

[1] Zheng, S., Xin, L., Liang, A., Fu, Y. *Cancer stem cell hypothesis: a brief summary and two proposals*, Cytotechnology 65 (2013): 505-512.

[2] Helmlinger, G., Netti, P.A., Lichtenbeld, H., Melder, R., Jain, R.J. *Solid stress inhibits the growth of multicellular tumor spheroids*, Nature Biotechnol. 15 (1997): 778-783.

[3] Montel, F. et al. *Stress Clamp Experiments on Multicellular Tumor Spheroids*, Phys. Rev. Lett. (2011) 107, 188102: 1-4.

[4] Van Liedekerke, Paul et al. *Quantitative agent-based modeling reveals mechanical stress response of growing tumor spheroids is predictable over various rowth conditions and cell lines*, PLoS Comput. Biol. 15(3) (2019): e1006273.

[5] Benítez, L, Barberis, L. Condat, C.A. *Modeling tumorspheres reveals cancer stem cell niche building and plasticity*, Physica A 533 (2019), 121906: 1-11.

[6] Chen, Y.C. et al. *High-throughput single-cell derived sphere formation for cancer stem-like cell identification and analysis*, Sci. Rep. 6 (2016), 27301: 1-12.