

Ontogenetic growth of multicellular tumor spheroids

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Available online 19 May 2006

Abstract

In ontogenetic growth models, the basal metabolic rate is usually assumed to depend on the individual mass following a power law. Here it is shown that, in the case of multicellular tumor spheroids, the emergence of a necrotic core invalidates this assumption. The implications of this result for spheroid growth are discussed, and a procedure to determine the growth parameters using macroscopic measurements is proposed.

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Keywords: Ontogenetic growth; Diffusion; Necrosis; Multicellular tumor spheroid; Cancer

1. Introduction

The process of the individual development of an organism is called ontogenesis. The existence of universal features characterizing ontogenetic growth has been suspected for many years. In a seminal 2001 paper, West, Brown, and Enquist (WBE) showed that two hypotheses suffice to ensure universality: the conservation of energy and the fractality of the nutrient distribution network [1]. In 2003 Guiot and co-workers suggested that cancer should also follow the same growth pattern [2]. Later on, WBE's result was generalized to include nutrient diffusion as the determining factor controlling growth. This is the case with multicellular tumor spheroids, which are experimental systems widely used to model many aspects of cancer growth and therapy [3]. The case for using WBE's theory was strengthened by its application to spheroids growing under conditions of starvation and increased medium rigidity [4] and by the bridging between mesoscopic and macroscopic models of cancer growth achieved with the introduction of an "intermediate model" [5].

Even under ideal conditions, spheroids whose diameter exceeds about 0.8 mm develop a necrotic core, because oxygen and nutrients cannot get beyond a viable outer shell. After the emergence of the necrotic core, the spheroid continues to grow, but the thickness of the live cell layer remains constant. A limitation of the model of Ref. [4] is that it was restricted to homogeneous spheroids. The purpose of this paper is to generalize it to spheroids containing a necrotic core.

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2. The model

Following WBE's ideas [1], we start from the energy conservation equation, which states that the energy income rate to the organism cells equals the energy used for the maintenance and replacement of existing cells plus the energy devoted to the creation of new cells. If dN cells are created during a time interval dt , the energy conservation equation is,

$$B dt = N \beta dt + \varepsilon dN, \quad (1)$$

where β and ε are, respectively, the single cell metabolic rate and the energy required to create a single cell, N is the total cell number, and B is the energy income rate to the organism cells. Defining $b = \beta/\varepsilon$, we can write a differential equation for the total organism mass, m , where $m = Nm_c$, and m_c is the average cell mass,

$$\frac{dm}{dt} = \frac{m_c}{\varepsilon} B(m) - bm. \quad (2)$$

Here the basal metabolic rate $B(m)$ must be modeled. In Ref. [4], $B(m) = B_0 m^p$ was chosen, with $p = \frac{3}{4}$ corresponding to fractal nutrient distribution and $p = \frac{2}{3}$ to diffusive nutrient distribution. In the case of spheroids that never develop necrosis, which we shall call *type-I* spheroids, we must adopt $p = \frac{2}{3}$. As a consequence, the spheroid grows up to a maximum mass $M = (a/b)^3$, where $a = m_c B_0/\varepsilon$. The time dependence of the spheroid mass is given by

$$m(t) = M(1 - e^{-bt/3})^3. \quad (3)$$

What happens if a necrotic core emerges? A crucial point is the realization that once the necrotic process has begun, the thickness Δ of the live cancer cell shell usually remains approximately constant. This feature had been already predicted by Burton in 1966 [6] and it has been reviewed by Mueller-Klieser [3] (see Fig. 1). Since the mass appearing in Eq. (2) is the *live cell* mass, these *type-II* spheroids exhibit two well-defined growth regimes: at first, the spheroid follows the $p = \frac{2}{3}$ law described above, but after a time τ defined by $m(\tau) = \hat{m} \equiv (\frac{4}{3})\pi\rho\Delta^3$, the live cell mass is related to the spheroid radius r through the equation $m(t > \tau) = \rho V_c$, where V_c is the volume occupied by live cells and ρ is the mass density. A simple calculation yields,

$$r(m) = \frac{\Delta}{2} \left[1 + \frac{1}{\sqrt{3}} \left(4 \frac{m}{\hat{m}} - 1 \right)^{1/2} \right]. \quad (4)$$

At times $t > \tau$, the necrotic core diameter D_N satisfies the equation $D_N = D - 2\Delta$, with $D = 2r$. Fig. 1 shows an example where this equation is precisely satisfied. Since diffusion is still the rate-limiting process for nutrient

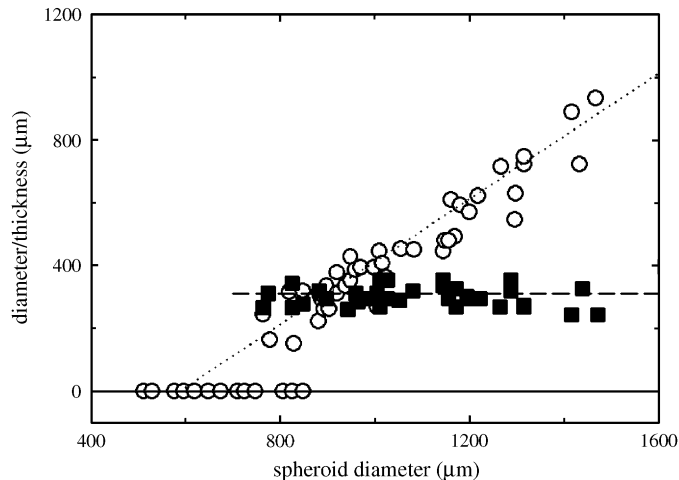


Fig. 1. Viable rim thickness (squares) and necrosis diameter (circles) in spheroids of *myc/ras*-transfected rat embryonic fibroblasts as functions of spheroid diameter. The dotted line has slope unity (experimental data from Ref. [3], with permission).

arrival, B is still proportional to r^2 . Therefore, the live cell mass evolves following the equations,

$$\frac{dm}{dt} = \begin{cases} am^{2/3} - bm & \text{if } m < \hat{m}, \\ \frac{a\hat{m}^{2/3}}{12} \left[\left(4\frac{m}{\hat{m}} - 1 \right)^{1/2} + \sqrt{3} \right]^2 - bm & \text{if } m > \hat{m}. \end{cases} \quad (5)$$

Although these equations can be solved exactly, the detailed analytical form of the time-dependent solution is not particularly illuminating. By looking at the possible steady-state solutions, we find that the spheroid evolution at times $t > \tau$ depends on the relative sizes of M and \hat{m} :

- (a) *Unlimited growth*: If $\hat{m} < (\frac{1}{27})(a/b)^3 = (\frac{1}{27})M$, the live cell shell is very thin and there is always an excess energy that leads to continuous growth.
- (b) *Convergence to a steady state*: If $(\frac{1}{27})M < \hat{m} < M$, the live cell mass grows monotonically until it reaches a steady-state value m_s^II ,

$$m_s^II = \frac{3}{2(1-3Q)^2} \left(\frac{2}{3} + Q + Q\sqrt{\frac{4}{Q} - 3} \right) \hat{m}. \quad (6)$$

Here $Q = (\hat{m}/M)^{1/3} \leq 1$. The steady-state mass grows from $m_s^II = \hat{m}$ when $Q = 1$ to $m_s^II = \infty$ when $Q \rightarrow (1/3)^+$.

- (c) *Homogeneous spheroid*: Of course, if $M < \hat{m}$, we have no necrotic core, Eq. (4) never applies and the steady state is given by

$$m_s^I = M = (a/b)^3. \quad (7)$$

3. Discussion

By concentrating resource utilization in the outer rim, the emergence of necroses helps spheroids to reach larger sizes. However, oxygen and nutrients always penetrate deep enough to ensure that condition (a) above is never reached, and maximum spheroid diameters remain usually below 2 mm. In vivo tumors often develop necrotized regions that are likely to favor continuous growth until angiogenic development ensures tumor thriving. Our calculations indicate that, except for its very early stages, tumor growth will not be controlled by a pure power law, but will have a more complex form. Eq. (4) suggests that the function $B(m)$ corresponding to a tumor containing a large necrosis may have a stronger dependence on the mass than the $B(m) \sim m^{3/4}$ characteristic of a fractal nutrient distribution network. In this connection, we note the recent introduction of a “dynamical exponent” to characterize tumoral growth [7].

Our understanding of growth dynamics would be enhanced if we can determine the values of the parameters a and b for various tumor cell species. We propose that these parameters may be estimated by performing macroscopic measurements on multicellular tumor spheroids. This could be done as follows: the final radius for a *type-I* spheroid is given by

$$R = \left(\frac{3}{4\pi\rho} \right)^{1/3} \left(\frac{a}{b} \right). \quad (8)$$

Therefore, the ratio of the parameters determining spheroid growth can be obtained by a simple geometric measurement. According to Eq. (3), the value of b can be independently ascertained by plotting the function $-3 \ln[1 - (m/M)^{1/3}]$ versus t and determining the slope.

In the case of *type-II* spheroids, we must determine three parameters. While Δ is experimentally accessible, the procedure to determine a and b is more cumbersome than for a *type-I* spheroid. Although Eq. (6) can be used, large inaccuracies may follow. Perhaps the best method is to determine b as in the case of *type-I* spheroids, using $t < \tau$ data, and then measure the necrosis onset time τ . Since $m(\tau) = \hat{m}$, the parameter a can be

determined from

$$a = \left(\frac{4\pi\rho}{3}\right)^{1/2} \Delta b(1 - e^{-b\tau/3}). \quad (9)$$

The availability of reliable estimates for these parameters would set useful constraints on cancer growth models.

Acknowledgments

This work was supported by CONICET, through PIP 6311/05, and by SECyT-UNC.

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