A computational model for telomere-dependent cell-replicative aging

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Abstract

Telomere shortening provides a molecular basis for the Hayflick limit. Recent data suggest that telomere shortening also influence mitotic rate. We propose a stochastic growth model of this phenomena, assuming that cell division in each time interval is a random process which probability decreases linearly with telomere shortening. Computer simulations of the proposed stochastic telomere-regulated model provides good approximation of the qualitative growth of cultured human mesenchymal stem cells.

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1. Introduction

Telomere are specialized nucleoproteins involved in protection and stabilization of chromosomes ends. They contain tandem repetitive arrays of DNA which in vertebrates is the sequence (TTAGGG)\textsubscript{n}. The overall human telomere sizes range from \(\approx 15\) kb at birth to less than 5 kb at chronic disease states (Shay and Wright, 2005). With cell division, telomere lose TTAGGG repeats mainly by incomplete replication of linear chromosomes by conventional DNA polymerases (Greider, 1996). So, most of somatic cells experience progressive telomeric shortening with cell division.

There are compelling evidences that cells count past divisions, and that maturation and aging depends on the number of such divisions. The most likely counting mechanisms appears to be telomere shortening. There are also evidences that bellow a certain telomere-length cells cease to divide (replicative senescence) (Shin et al., 2006). This mechanism provides a good explanation for the Hayflick limit (Hayflick, 1965).

Recent in vitro experiments has shown that telomere shortening correlates negatively with cell proliferation in cultured mesenchymal cells. In this setting, there is a gradual decrease both in telomere length and population-doubling rate (Baxter et al., 2004; Bonab et al., 2006). Furthermore, reduction in telomeric length was found to be linearly correlated with the proliferative capacity of cells (Gupta et al., 2007).

In vivo research also indicates that telomere shortening negatively affects cellular proliferation. This phenomena has been verified in recent study on mice tumoral growth. Moreover, this effect was also observed.
under apoptosis inhibition, as described in Feldser and Greider (2007). These results were long awaited and may have important implications on future cancer therapy (Zimmermann and Martens, 2007; Sedivy, 2007).

Our main purpose is to corroborate telomere-dependent replicative aging by a stochastic model and computer simulations. Cell division is regarded as a random process which probability decreases linearly with telomere shortening, yielding a stochastic telomere-regulated growth model. Computer simulations of this model match recent data from cultured human mesenchymal stem cells (hMSCs). Human MSCs can be readily isolated from bone marrow and expanded in culture (Caplan and Bruder, 2001). They have attracted much attention because of their regenerative potential, since these cells can be differentiated into chondrogenic, osteogenic, adipogenic and miogenic lineages (Pansky et al., 2007).

Deterministic Gompertzian growth provides a very good fitness for somatic (Laird, 1965; Laird et al., 1965; Begall, 1997) and tumoral growth (Laird, 1969; Norton, 1988; Sullivan and Salmon, 1972; Demichelis, 1980). The stochastic telomere-regulated model presented in this work also provides a good approximation to Gompertzian growth, both in computer simulations and theoretical ground.

2. A stochastic telomere-regulated growth model

It is well known (Lin and Yan, 2005) that a cell can not divide if its telomere is shorter than some minimal length. Very short telomere length correspond to crisis cell (Greider, 1996). It is also known that cells lose 30–150 bases of telomere in each cell division (Lin and Yan, 2005). In view of this facts, we will build our model considering the following assumptions:

A1. There is an initial cell population of \( N_0 \) cells, all of them having the same telomere length \( L_0 \).

A2. At each cell division, the telomere decreases a fixed amount \( \delta > 0 \).

A3. Cell division does not occur for a telomere length \( L \leq L_{\text{min}} \), where \( L_{\text{min}} \geq \delta \).

For the sake of simplicity, time is discretized. In this discrete time model, cell division is a random process which is independent for each cell and depends only on telomere length. Motivated by in vitro and in vivo evidences that telomere shortening negatively affects cellular proliferation (Baxter et al., 2004; Bonab et al., 2006; Feldser and Greider, 2007), we assume that the division probability for each cell decreases as a result of the shortening of the telomere. As previously discussed, telomere shortening seems to be linearly correlated with the proliferative capacity of cells (Gupta et al., 2007). So, we assume that the dependence of division probability on telomere length is linear:

A4. For a cell of telomere length \( L \in [0, L_0] \), probability of division at each moment \( p(L) \) is given by

\[
p(L) = \begin{cases} 
\alpha(L - L_{\text{min}}), & L > L_{\text{min}}, \\
0, & \text{otherwise}
\end{cases}
\]

with \( 0 < \alpha \leq (L_0 - L_{\text{min}})^{-1} \).

Note that making \( \alpha \to 0 \) one retrieves as a limit after normalizing, a continuous time model.

According to A1 and A2, a cell which has undergone \( k \) divisions would have a telomere length \( L(k) \):

\[
L(k) = L_0 - k\delta.
\]

So, from A3 we conclude that the maximum number of past cell divisions counting from the initial population is

\[
k_{\text{max}} = \left\lceil \frac{L_0 - L_{\text{min}}}{\delta} \right\rceil
\]

where \( \lceil x \rceil \) stand for the smallest integer greater or equal to \( x \), the ceiling function.

Probability of division can also be expressed as a function of the number of past cell divisions \( k \) counted from the original cell population:

\[
p(k) = \begin{cases} 
\alpha(L_0 - L_{\text{min}} - k\delta), & 0 \leq k < k_{\text{max}}, \\
0, & \text{otherwise}
\end{cases}
\]

For cells with telomere length \( L > L_{\text{min}} \), the mean time to (next) division is

\[
\bar{t} = \mathbb{E}(t|L) = \frac{1}{p(L)} = (\alpha(L - L_{\text{min}}))^{-1}.
\]

If at some time \( t \) a cell has a telomere length \( L > L_{\text{min}} \), then the probability of this cell to suffer a division in the time interval \( [t, t+u] \) goes to one as \( u \to \infty \). In other words, division is sure to occur in all cells with telomere length strictly greater than \( L_{\text{min}} \).

The total number of cells at time \( t \in \mathbb{N} \) is a random variable \( N(t) \). Since division is sure for telomere length strictly greater than \( L_{\text{min}} \), a final size of \( N_0 2^{k_{\text{max}}} \) is reached with certainty:

\[
\lim_{t \to \infty} P(N(t) = N_0 2^{k_{\text{max}}}) = 1.
\]
We are concerned with the behavior of particular instances of growth curves and also with the mean-size (expected size) growth curve. At any time \( t \), let \( N(k, t) \) be the number of cells which has undergone \( k \) divisions, counting from the initial population, \( k = 0, \ldots, k_{\text{max}} \). Then
\[
N(k, 0) = \begin{cases} N_0, & k = 0 \\ 0, & k = 1, \ldots, k_{\text{max}} \end{cases}
\]

At any time \( t \in \mathbb{N} \):
\[
N(t) = \sum_{k=0}^{k_{\text{max}}} N(k, t).
\]

Cells in the subpopulation \( N(k_{\text{max}}, t) \) never divide. Let us call \( D(k, t) \) the number of cells, in the subpopulation \( N(k, t) \), which divide at time \( t \) (i.e. from \( t \) to \( t + 1 \)). Then, \( D(k, t) \) is a random variable with a (conditional) binomial distribution:
\[
P(D(k, t) = d | N(k, t) = n) = \begin{cases} \binom{n}{d} p(k)^d (1 - p(k))^{n-d}, & 0 \leq d \leq n, \\ 0, & \text{otherwise}. \end{cases}
\]

We ran a simulation of this model in MATLAB R12 using as pseudo-random number generator the function \texttt{randraw}, available at the “File Exchange” web site of Matlab Central.\footnote{http://www.mathworks.com/matlabcentral/}. In Fig. 1 is plotted the mean cumulative population doubling (PD) obtained from the computer simulation of the stochastic growth model.

In this simulation we used the following values of the parameters:
\[
N_0 = 10^3 \text{cells}, \quad L_0 = 12.2 \text{ kb},
\]

\[ L_{\text{min}} = 10.4 \text{ kb}, \quad \delta = 86 \text{ bp}, \quad \alpha = 1/3000 \quad (4) \]

where \( \Delta t \) is the (discrete) time unit. Here, the maximum number of mitosis is 21. Is interesting to observe that this curve is qualitatively similar to experimental data of cultured human marrow stromal cells, presented in Baxter et al. (2004).

In Fig. 2 the stochastic model with parameters (4) is plotted, together with the best fitting Gompertzian growth with the same initial and final population. The fitting is quite good and possible explanations are discussed in the next two sections.

3. **Gompertzian growth as an ODE approximation of the stochastic model**

In a continuous time model, assumption A4 must be replaced by an exponential distribution model:

\[ A4c. \quad \text{For a cell of telomere length } L \in [0, L_0] \text{ at time } t, \text{ division at } \tau \geq t \text{ is a random process with an exponential distribution:} \]
\[
p(\tau) = \lambda(L) \exp(-\lambda(L)(\tau - t)), \quad \tau \in [t, \infty)
\]

where
\[
\lambda(L) = \alpha(L - L_{\text{min}}).
\]

For a cell with with telomere length \( L > L_{\text{min}} \), the mean time to divide is
\[
\bar{T} = \frac{1}{\lambda(L)} = (\alpha(L - L_{\text{min}}))^{-1}.
\]

Hence, for a large cell population \( N \) with telomere length \( L > L_{\text{min}} \) at \( t_0 \):
\[
\frac{dN}{dt} = \lambda(L) N = \alpha(L - L_{\text{min}}) N.
\]
After $k$ synchronized division cycles of the initial population, without apoptosis, the overall number of cells would be

$$N = N_0 2^k,$$

and then, $k = \log_2 N/N_0$.

Of course, in large cell population, cell divisions shall not be synchronized. Then, at any time $t$, the mean number of past divisions $\bar{k}(t)$ for each cell in the population is roughly proportional to the logarithm of the total number of cells, that is

$$\bar{k}(t) \approx \log_2 N/N_0. \quad (5)$$

Here, if $c_1, c_2, \ldots, c_N$ denotes the individual cells and $k_1, k_2, \ldots, k_N$ are the respective number of past divisions, then

$$\bar{k}(t) = \frac{1}{N} \sum_{i=1}^{N} k_i.$$

Using estimation (5), at any time $t$ we have

$$\bar{k}(t) = \log_2 N - \log_2 N_0.$$

Combining the above equation with (1), we obtain for the mean telomere length in the cell population, $\bar{L}(t)$:

$$\bar{L}(t) \approx L_0 - \delta \bar{k}(t) = (L_0 + \delta \log_2 N_0) - \delta \log_2 N(t).$$

Now using Assumption A4c and approximating the telomere length of each cell of the population by its mean value $\bar{L}(t)$, we have

$$\frac{1}{N} \frac{dN}{dt} = C \left( \bar{L}(t) - L_{\text{min}} \right),$$

using also the previous estimation for $\bar{L}(t)$ we have

$$\frac{1}{N} \frac{dN}{dt} = \delta C \left[ \left( \frac{L_0 - L_{\text{min}}}{\delta} + \log_2 N_0 \right) - \log_2 N \right]. \quad (6)$$

Using the natural logarithm instead of $\log_2$ in this equation we obtain:

$$\frac{1}{N} \frac{dN}{dt} = \delta C \left[ \ln \left( N_0 2^{(L_0 - L_{\text{min}})/\delta} \right) - \ln N \right], \quad (7)$$

which describes the Gompertzian growth (Gompertz, 1825; Laird, 1965).

4. From the deterministic to the stochastic model

In the stochastic model, the inverse of the expected time interval between successive mitosis is the probability of mitosis in a time unit. Therefore, it is relevant to investigate this magnitude in a discrete deterministic model which follows a Gompertzian dynamics. Here, discrete is related to the size of cell population, which shall be integer. This is the aim of this section. We will show that for such a deterministic dynamics, the inverse of the time interval (between successive mitosis) is very well approximated by an linear (affine) function. Therefore, a stochastic approximation of this discrete deterministic Gompertz-like dynamics shall have a division probability which decrease linearly with the past mitosis number as in (3).

Let us assume that a cell population of $N_0$ cells undergoes simultaneously mitosis up to a number of $k_{\text{max}}$ consecutive mitosis at time:

$$0 < t_1 < t_2 < \cdots < t_{k_{\text{max}}}.$$

Defining $t_0 = 0$, at any time, the population is then

$$N(t) = \begin{cases} 2^k N_0 & t_{k-1} < t < t_k, \quad k = 1, 2, \ldots, k_{\text{max}} \\ 2^k_{\text{max}} N_0 & t_{k_{\text{max}}} \leq t. \end{cases} \quad (8)$$

Equivalently,

$$\log_2 \left( \frac{N(t)}{N_0} \right) = \begin{cases} k & t_{k-1} < t < t_k, \quad k = 1, 2, \ldots, k_{\text{max}} \\ k_{\text{max}} & t_{k_{\text{max}}} \leq t. \end{cases} \quad (9)$$

A Gompertzian model of such population $\tilde{N}(t)$ will be given by

$$\log_2 \left( \frac{\tilde{N}(t)}{N_0} \right) = k_{\text{max}} (1 - e^{-\beta t}). \quad (10)$$

At time $t_k$, $k \geq 1$, the cell population $N(t)$ jumps from $2^{k-1} N_0$ to $2^k N_0$ cells. Hence, around $t_k$, the mean value of $\log_2(N/N_0)$ is approximately

$$k - 1 = \frac{(k - 1) + k}{2} = \frac{k}{2}.$$

Hence, a good fit between the step function $N$ and the continuous function $\tilde{N}$ can be obtained if we assume

$$\log_2 \left( \frac{\tilde{N}(t_k)}{N_0} \right) = \frac{k - 1}{2}, \quad k = 1, 2, \ldots, k_{\text{max}}.$$

In Fig. 3 the Gompertzian dynamics and the discrete deterministic model for this choice of $t_k$ are plotted together. Combining the above condition with (10) we obtain:

$$t_k = \beta^{-1} \log \left( \frac{k_{\text{max}}}{k_{\text{max}} (k - 1)/2} \right) \quad k = 1, 2, \ldots, k_{\text{max}}. \quad (11)$$
Therefore, the time interval for the $k$th mitosis is

$$\Delta t_k = t_k - t_{k-1} = \frac{1}{\beta} \log \left(1 + \frac{1}{k_{\text{max}} - k + 1/2}\right) \quad k = 2, \ldots, k_{\text{max}}.$$  \hfill (12)

In a stochastic model with probability $\pi(k)$ of the $k$th mitosis in each time interval, the mean time interval to the $k$th mitosis is

$$\Delta t_k = \frac{1}{\pi(k)}.$$

Therefore, to get concordance with (12) we shall have

$$\pi(k) = \frac{\beta}{\log (1 + (1/k_{\text{max}} - k + 1/2))}.$$

The function $\pi(k)$ was modeled by an affine function in the telomere-regulated stochastic model (see Eq. (3)). It happens that this approximation is quite good. Indeed, for $k_{\text{max}} = 28$, consider $f$ the affine approximation:

$$f(k) = \pi(2) + k \frac{\pi(28) - \pi(2)}{28 - 2}.$$

The relative error (which does not depend on $\beta$):

$$\left|\frac{\pi(k) - f(k)}{\pi(k)}\right|, \quad k = 2, \ldots, 28$$

has a maximum value of 2% and a mean value of 0.5%.

5. Discussion

In the current work we presented a model of how telomere shortening correlates with growth and replicative senescence. Somatic growth is a complex biological phenomenon that is controlled by many mechanisms. Culture of cell offers a simplified setting for the study of growth and replicative aging. The growth pattern of cultured human stromal cells was qualitatively similar to the process generated by our stochastic model. We assume that telomere shorten at each cell division and no telomerase activity is present. The output of the model fits with data of cultured bone marrow stromal cells where telomerase activity is not detected (Zimmermann et al., 2003). Mathematical models for telomere shortening has already been proposed. However, these models usually described telomere shortening as an autonomous process, measures telomere length distributions and do not correlate this with cellular growth and Gompertzian growth (Arino et al., 1995; op den Buijs et al., 2004; Proctor and Kirkwood, 2002).

Somatic and tumoral growth and also regeneration are well model by Gompertzian curve. In the simulation of the stochastic model we obtained a very good approximation of Gompertzian growth. Theoretical explanations for this finding indicate that Gompertzian growth can be derived as an continuous time approximation of the stochastic model. In the other way around, we observe that a stochastic model which approximate Gompertzian growth must have a (almost) linear decreasing probability of cell division. This is in accordance with the fourth assumption (A4) of the stochastic model. This finding may provide a clue on the biological foundations of Gompertzian growth.

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References


