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Mathematical Models Predicting Clonal Senescence of WI-38 Cells[1]

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It is the purpose of this article to show that, under very general assumptions, the observed senescence of diploid cells in culture cannot be accounted for by assuming a constant production of any kind of "damage." It follows that other factors must play a role in cell senescence or that the damage is autocatalytic.

Key Words: Growth

SINCE the discovery that diploid cells in culture have a finite life span (Hayflick & Moorhead, 1961), there has been a great deal of research into this phenomenon (Cristofalo & Sharf, 1973; Deamer & Gonzales, 1974; Hayakawa, 1969; Hayflick, 1965; Machiera-Coelho et al., 1966; Merz & Ross, 1969; Smith & Hayflick, 1974; Smith & Lumpkin, 1980; Smith & Whitney, 1980). In addition to the experimental work there have been attempts to provide a mathematical model of the phenomenon (Good, 1977; Grove & Cristofalo, 1976; Hahn, 1966; Hurley, 1975; Jones et al., 1980; Smith & Martin, 1973, 1974). Several models assume that the mechanism controlling the growth rate of WI-38 cells reflects accumulating "damage" of some form in the cells. Some assume, in addition, that the growth rate is extremely sensitive to the amount of damage present.

The purpose of this article is to demonstrate that, under fairly general assumptions, it is impossible to account for the observed senescence as an effect of a single kind of accumulating damage alone. To be somewhat more precise, this model is general enough to deal with the effects of any kind of accumulating effect that is not genetically programmed. Peroxidative damage and somatic mutations are probably the best known examples of the type of effect that we are considering. The model is not able to deal with genetic effects—either as directly controlling senescence or indirectly by means of genetically programmed damage. The various effects considered by this article will be lumped under the general term "damage."

Intuitively speaking, the reason that damage alone will not account for the observed senescence (under the assumptions stated later) is that the damage in cells attains its maximum level very quickly (after 10 generations) and does not change much after that. Thus, the damage present in cells of generation 10 ought to be virtually the same as that in cells of generation 40. But this is known to be false and, moreover, the cells themselves behave very differently. Expressing these ideas in precise form constitutes the bulk of this article. Alternative explanations are posed at the end.

Assumptions Underlying Our Models

We divide our assumptions into two general categories. The first category consists of those assumptions that seem to be generally accepted.

A1. Biological systems in general are not very sensitive to small proportional differences in cytoplasmic damage.

A2. Embryonic cells contain negligible damage.

A3. All damage in a cell at the time of mitosis is divided between the two daughter cells (although not necessarily equally).

The three other assumptions are specific to this model. The first two seem plausible but need to be tested. It is the third that we are trying to show is false.

B1. The proportional distribution of damage between the two daughter cells does not depend on the amount of damage present.

B2. No more than 10 times the average amount of damage produced per generation is produced in any one generation (i.e., cells do

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not occasionally produce vast amounts of damage, whereas the rest of the time produce little).

B3. Net damage occurs at a constant rate in each generation regardless of the age of the cell or the amount of damage already present.

In the last assumption the term "net damage" was chosen with the underlying idea that there may be independent mechanisms that both create and repair damage. It does not matter to the model nor is it particularly important whether or not we assume that the net damage accrues at a constant rate per generation or a constant rate per hour. The intermitotic times are sufficiently constant for many generations to make the two assumptions interchangeable.

Our basic strategy is to show that, under the six assumptions listed, the damage distribution of a cell at generation 25 is essentially identical with that for all subsequent generations. If assumption A1 is true, then the cells should behave the same. But, since cells of generation 25 are known to grow very differently from those of generation 40, it will follow that one of our set of assumptions is in error. As assumptions A1 to A3 are more likely to be valid, either assumption B1, B2, or B3 must be false.

Mathematics of Model

Let $X_n(s)$ represent the probability distribution of damage present in the cells as a function of n, the number of generations. That is, $X_n(s)$ denotes the probability that a cell contains an amount of damage $\leq s$ at the beginning of generation n. As mentioned before we wish to show that $X_n(s)$ is virtually constant when $n \geq 25$.

The damage distribution $X_n(s)$ depends, in turn, on two other distributions. $D(z)$ is the probability that a cell in one generation produces an amount of damage $\leq z$ (note that this distribution is independent of n and the amount of damage present by assumption B3). We suppose that $D(z)$ has mean d and standard deviation σ_d but make no assumption that the distribution is normal.

We also need a probability distribution for the dispersion of the damage between the two daughter cells at mitosis. According to assumption A3 all damage is inherited by one of the two daughter cells. According to assumption B1 this distribution function does not depend on the amount of damage present. Let $Q(y)$ represent the probability that the first daughter cell inherits a *fraction* of the damage in the parent cell $\leq y$. By symmetry, $Q(y) + Q(1 - y) = 1$, and so the mean of $Q(y)$ is ½. Moreover, if *all* of the damage in a parent cell always went to one daughter cell, the standard deviation would be ½. Thus, we may conclude that σ_Q, the standard deviation of Q, is $\leq$ ½.

We wish to compare $X_{25}(s)$ and $X_{40}(s)$ to show that they are virtually identical. In fact we will conclude that $X_n(s)$ has essentially the same distribution as $X_{25}(s)$ for all $n \geq 25$. (If we assume that the D and Q distributions are near normal, then the $X_n(s)$ converge much sooner.)

Our approach is to begin with an arbitrary embryonic cell and follow the damage distribution in one daughter cell of each generation. By conceptually following a large number of daughter cells we may arrive at the damage distribution within the cells of any generation.

Let $x_0(n)$ denote the amount of damage present within our specified daughter cell at the beginning of generation n. Let $x_1(n)$ denote the amount of damage present in that cell at the end of generation n. (By definition, the set of $x_0(n)$ give rise to X_n.) Let d_n be the amount of damage created within the cell during generation n and q_n is the fraction of the total damage within the parent cell carried over to the specified daughter cell in generation $n + 1$.

If we assume that our embryonic cell begins with an amount of damage ϵ, then for the first three generations we have

$$x_0(0) = \epsilon$$

$$x_1(0) = d_0 + \epsilon$$

$$x_0(1) = q_0d_0 + q_0\epsilon$$

$$x_1(1) = d_1 + q_0d_0 + q_0\epsilon$$

$$x_0(2) = q_1d_1 + q_1q_0d_0 + q_1q_0\epsilon$$

$$x_1(2) = d_2 + q_1d_1 + q_1q_0d_0 + q_1q_0\epsilon$$

In general, we have

$$x_0(n) = q_{n-1}d_{n-1} + q_{n-1}q_{n-2}d_{n-2} + \cdots + q_{n-1}q_{n-2}\cdots q_0d_0 + q_{n-1}q_{n-2}\cdots q_0\epsilon$$

Note that by assumption B1 all of the q_i are from distribution Q and by assumption B3 all of the d_i are from distribution D. So, if we look at the aggregate of all possible daughter cells and their damage distribution, it follows that

$$X_n = QD + Q^2D + \cdots + Q^nD + Q^n\epsilon$$

Note that if $m > n$ the first n terms of this equation are just those of X_m. So we may write

$$X_m = X_n + Q^n X_{m-n} - Q^n \epsilon \tag{1}$$

In particular we have $X_{40} = X_{25} + Q^{25}X_{15} - Q^{25}\epsilon$. Measuring the difference between X_{40} and X_{25} means measuring the effect of the "tail": $Q^{25}X_{15} - Q^{25}\epsilon$. Since ϵ is assumed to be small (by assumption A2), we will ignore the term involving it and concentrate on the other.

We will demonstrate that

$$\mu(Q^{25}X_{15}) = \mu(Q)^{25}\mu(X_{15}) \leq \frac{1}{2^{25}}d \approx .000001d$$

So the mean is vanishingly small. All we need to verify is that the variance $\sigma^2(Q^{25}X_{15})$ is also small. This requires some work and will be done later.

Using the previous results, however, we have

$$P\left(Q^{25}X_{15} \geq \frac{d}{100}\right) \leq .006. \tag{2}$$

That is, the probability that the tail changes the distribution by more than 1% of the average damage is $\leq$.006. Stated in other terms, only six times out of 1,000 would we expect the damage distributions of cells of generation 25 and cells of generation 40 to differ by more than 1%.

So the entire damage distribution of cells of generation 25 and generation 40 (and not just their means) are virtually identical 99% of the time. But since the cells behave differently, some other factor must be at work. This concludes the argument except for the rather tedious calculations of the next section.

Bounding the Variance of the Tail

We need the following standard facts for independent random variables A and B (where μ denotes mean and σ^2 denotes variance).

$$\mu(A + B) = \mu(A) + \mu(B) \tag{3}$$

$$\sigma^2(A + B) = \sigma^2(A) + \sigma^2(B) \tag{4}$$

$$\mu(AB) = \mu(A)\mu(B) \tag{5}$$

$$\begin{aligned} &\sigma^2(AB) + \mu^2(AB) \\ &\quad = [\sigma^2(A) + \mu^2(A)][\sigma^2(B) + \mu^2(B)] \end{aligned} \tag{6}$$

Recalling that $\mu(Q) = \frac{1}{2}$ and $\mu(D) = d$, from Equations 1, 3, and 5, we see that

$$\begin{aligned} \mu(X_n) &= \frac{1}{2}d + \left(\frac{1}{2}\right)^2 d + \cdots + \left(\frac{1}{2}\right)^n d\left(\frac{1}{2}\right)^n \epsilon \\ &= d\left(1 - \frac{1}{2^n}\right) + \frac{\epsilon}{2^n} \end{aligned} \tag{7}$$

Again, since assumption A2 asserts that ϵ is negligible, our last equation says that for $n \geq 10$, $\mu(X_n)$ lies in the range .999d to 1.000d, so the means converge very quickly to a fixed value virtually equal to the average amount of damage produced in one generation.

Let $\tau(S)$ denote the sum $\sigma^2(S) + \mu^2(S)$ for any random variable S. Then, by induction on Equation 6 we see that

$$\tau(Q^n) = \tau^n(Q) \tag{8}$$

Thus, again using Equation 6

$$\tau(Q^n X_p) = \tau^n(Q)\tau(X_p) \tag{9}$$

and, hence

$$\sigma^2(Q^n X_p) = \tau^n(Q)\tau(X_p) - \mu^{2n}(Q)\mu^2(X_p) \tag{10}$$

In particular, Equation 9 tells us that

$$\begin{aligned} \tau(Q^n X_{15}) &= \tau^n(Q)\tau(X_{15}) \\ &\leq [\sigma_Q{}^2 + (\tfrac{1}{2})^2]^n \tau(X_{15}) \leq \tau(X_{15})/2^n \end{aligned} \tag{11}$$

Thus, if we can bound $\tau(X_{15})$, we will have evaluated the variance we need.

It turns out to be simpler to bound $\sigma^2(X_{15})$. By Equations 1 and 4

$$\sigma^2(X_n) = \sigma^2(X_{n-1}) + \sigma^2(Q^{n-1}X_1) \tag{12}$$

Repeating this argument shows

$$\begin{aligned} \sigma^2(X_n) = \sigma^2(X_1) &+ \sigma^2(QX_1) \\ &+ \cdots + \sigma^2(Q^{n-1}X_1) \end{aligned} \tag{13}$$

So, by Equation 10

$$\begin{aligned} \sigma^2(X_n) &= \sum_{j=0}^{n-1} \tau^j(Q)\tau(X_1) - \mu^{2j}(Q)\mu^2(X_1) \\ &= \tau(X_1)\sum_{j=0}^{n-1}\tau^j(Q) - \mu^2(X_1)\sum_{j=0}^{n-1}\mu^{2j}(Q) \end{aligned}$$

But since $\tau(Q) \leq \frac{1}{2}$, $\mu(Q) = \frac{1}{2}$, $\mu(X_1) = \dfrac{d}{2}$ and

$\tau(X_1) = \frac{d^2}{4} + \sigma_D{}^2$ it follows that

$$\sigma^2(X_n) \leq \left(\frac{d^2}{4} + \sigma_D{}^2\right)(2) - \frac{d^2}{4}\left[\frac{4}{3}\left(1 - \left(\frac{1}{4}\right)^n\right)\right]$$

and hence,

$$\sigma^2(X_n) \leq 2\sigma_D{}^2 + \frac{d^2}{2} - \frac{d^2}{3} = 2\sigma_D{}^2 + \frac{d^2}{6} \qquad (14)$$

Thus, by Equation 7

$$\begin{aligned}\sigma^2(X_n) &\leq 2\sigma_D{}^2 + \frac{d^2}{6} + d^2\left(1 - \frac{1}{2^n}\right)^2 \\ &\leq 2\sigma_D{}^2 + \frac{7}{6}d^2\end{aligned} \qquad (15)$$

At this point it seems worthwhile to express $\sigma_D = \delta d$. Recall what these symbols mean. σ_D is the standard deviation of the damage production variable, and d is the mean. Thus δ measures the ratio of the standard deviation to the mean. Since d is in arbitrary units, we set $d = 1$. Since the random variable D always takes positive values (negative damage cannot be produced) and the mean is 1, δ cannot be too large in real cases.

In any event, Equation 15 may be rewritten as

$$\tau(X_n) \leq 2\delta^2 + \frac{7}{6} \qquad (16)$$

Hence,

$$\sigma^2(Q^{25}X_{15}) \leq \left(2\delta^2 + \frac{7}{6}\right) \Big/ 2^{25} \qquad (17)$$

At this juncture we apply Chebyshev's Inequality (Feller, 1957, p. 219). In our case, it is

$$P[Q^{25}X_{15} - \mu(Q^{25}X_{15}) \leq t] \leq \sigma^2(Q^{25}X_{15})/t^2$$

Or, in other terms: the probability that the difference between the variable of interest ($Q^{25}X_{15}$) and its mean exceeds the value t is $\sigma^2(Q^{25}X_{15})/t^2$.

Recalling that $\mu(Q^{25}X_{15}) \leq d/2^{25} = \frac{1}{2}^{25}$, this mean is so small we may safely ignore it.

Now apply Chebyshev's Inequality with $t = .01$ (using the fact $d = 1$):

$$P\left(Q^{25}X_{15} \geq \frac{d}{100}\right)$$

$$\leq \frac{1}{(.01)^2}\left[\left(2\delta^2 + \frac{7}{6}\right) \Big/ 2^{25}\right] \leq .0003\left[2\delta^2 + \frac{7}{6}\right]$$

It is at this point that we use assumption B2 that implies that $\delta \leq 3$. Hence, the probability that the tail is more than 1% of the average damage is $\leq .006$.

This is the fact used to establish Equation 2.

Conclusions

What possibilities remain as explanations of cell senescence? One, of course, is genetic, in any of a myriad of forms ranging from genetic determination of number of generations to some genetically programmed damage.

The other major hypothesis is that cells will reproduce in culture forever if some sort of damage is not produced that eventually interferes with the cells' reproductive mechanisms. If this damage occurs as an integral part of cellular activity, this article demonstrates that the damage cannot be produced at a constant rate (assuming assumption B1 is true).

Two other damage hypothesis possibilities remain that the author is investigating. First, the damage may be autocatalytic. A very simple mathematical model of autocatalytic damage fits some of the current data very well. It is hoped that a more sophisticated model will fit all of the known data.

The second possibility is that two (or more) separate damage factors interact in a currently unsuspected fashion to produce senescence.

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