Somatic selection of poorly differentiating variant stem cell clones could be a key to human ageing

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Abstract

Any replicating system in which heritable variants with differing replicative potentials can arise is subject to a Darwinian evolutionary process. The continually replicating adult tissue stem cells that control the tissue integrity of long-lived, multicellular, complex organisms, including humans, constitute such a replicating system. Our suggestion is that somatic selection for mutations (or stable epigenetic changes) that cause an increased rate of adult tissue stem cell proliferation, and their long-term persistence, at the expense of normal differentiation, is a major key to the ageing process. Once an organism has passed the reproductive age, there is no longer any significant counterselection to this inevitable Darwinian process.

Significance statement

When complex multi-tissue organisms pass the reproductive age, they suffer from increasing malfunction of many organs. There has so far been no overall satisfactory theory to explain this ageing process. Most adult tissues are formed by adult stem cells that persistently divide and produce differentiated daughter cells that form the mature tissue. These differentiated cells no longer divide, and eventually die by apoptosis, namely programmed cell death. We propose that, once an organism has passed the reproductive age there is greatly reduced counterselection against mutations, or stable epigenetic changes that affect gene expression. The tissue stem cell can then grow to its own advantage at the expense of the effect on the organism, and there is no longer significant selection for maintaining the organism's integrity. This "selfish" selective advantage of the adult tissue stem cell is mediated by a reduced ability to differentiate and by reduced apoptosis, leading to an increased growth rate of the stem cell but a reduced function.

Author Contributions

WFB conceived the project. WFB and DJMC wrote the manuscript.

Introduction

The major features of ageing in complex eukaryotes, notably extensive tissue malfunction as a result of the dysregulation of differentiation, have recently been extensively reviewed (for example, see [1-4]). There is much discussion of the genetic and epigenetic changes that could explain these ageing-associated changes, and the factors, including dietary influences, which could influence the rates at which these changes are produced and how the changes could be ameliorated. Adult tissue stem cells and the extent to which there is somatic selection in their evolution also figure in these discussions [5-7]. There is, however, hardly any mention of the possibility that the degenerative changes in ageing tissues could be the result of somatic clonal selection for genetic or epigenetic variants that, to an increasing extent, escape some of the constraints of differentiation and apoptosis and so lead to tissue malfunction.

It is widely accepted that cancer is a somatic evolutionary process in which the steps are genetic, or relatively stable epigenetic, changes, each step successively providing some advantage for the development and outgrowth of the cancer (for example, see [8]). For most cancers, a major step in this evolutionary process is escape from the control over the process by which they differentiate into cells with a programmed limited life span, and the most aggressive cancers are often those that have lost most of their capacity to differentiate. We suggest that cancer can thus be viewed as an extreme form of ageing, with respect to the tissue from which the cancer originates, and that similar violations of differentiation programs by adult stem cells are responsible for many normal ageing phenomena.

Any replicating system in which heritable variants with differing replicative potentials can arise is subject to a Darwinian evolutionary process, and these systems must include the continually replicating adult (i.e., not embryonic, pluripotent) tissue stem cells that control most of the tissue integrity of most relatively long lived, multicellular, complex organisms, including humans. As GC Williams [9] said in his much-quoted, perceptive

article on human ageing, "We are not machines that simply wear out, because our parts continually reproduce and refresh themselves throughout our lifetime". However, as Lopez-Otin et al. [4] said in their comprehensive review of 'The Hallmarks of Ageing', "Stem cell exhaustion unfolds as the integrative consequence of multiple types of ageing-associated damages and likely constitutes one of the ultimate culprits of tissue and organismal ageing" (for example, see also [10]). What is missing from this statement is an explanation of *why* all this damage leading to exhaustion should somehow accumulate disproportionately in the stem cells.

We propose that it is Darwinian somatic selection leading to the gradual dysregulation of the stem cell differentiation process and eventually to persistent tissue malfunction that is a major determinant of human ageing and its associated wide range of age-related diseases. This proposal is based on the following assumptions: 1) natural selection at the level of the whole organism effectively stops, or is at least greatly weakened, after reproduction is finished, 2) long term maintenance of most adult tissues is undertaken by adult tissue stem cells, but 3) after some period of time, adult tissue stem cells become exhausted and are then replaced by 'fresh' stem cells.

Background

It was first shown more than 20 years ago that normal human skin contains large numbers of clones of keratinocytes carrying TP53 mutations [11, 12]. Because these mutations are often due to thymine dimers, it was assumed that they were caused by UV in sunlight on exposed areas of the skin. The fact that the mutations are mostly missense mutations with functional effects strongly implies that the TP53 mutations give the clones a growth advantage, just like that found in many cancers and pre-cancerous growths, including colorectal adenomas. When there is a tumor adjacent to such a clone, it carries a different TP53 mutation, suggesting that in the cancer, the TP53 mutation is not a tumour initiating mutation but provides growth persistence (or immortalization) to the tumour (see e.g.,

[13]), after other mutations have been selected. Recently, a more extensive study of normal skin biopsies, using DNA sequencing, found evidence for frequent clones carrying a wide range of the sort of driver mutations found in cancers, notably those in NOTCH1, a gene whose function is closely tied to the maintenance of adult stem cells in epithelial tissues [14]. There is similar extensive evidence for somatic clonal selection in hematological cells from older individuals (see e.g. [7, 15-18]).

Weissmann's group showed that older mice have 5 times as many hematopoietic stem cells (HSCs) in their bone marrow as young or middle aged mice [19], but that these 'old' HSCs were much less efficient than 'young' HSCs at engrafting the bone marrow of irradiated mice. This clearly suggested somatic selection for clones that had lost much of their differentiating potential and as a result were faster and/or more persistent in their continued growth. More recently, it has become clear that elderly humans without cancers may have only a few dominant HSC clones in their normal bone marrow [20, 21] and that these HSCs often carry mutations in DNMT3A and TET2, genes that are key regulators of DNA methylation, which is the main epigenetic mechanism for gene expression control in differentiated cells. Mutations are also found in genes controlling hematopoiesis and in TP53 [3]. This is clear evidence for somatic selection of dominant clones that have outgrown their contemporaries. A comprehensive analysis of gene expression in highly purified HSCs from young and old mice showed increased promoter methylation associated with differentiation-promoting genes in the latter and decreased methylation with genes associated with HSC maintenance [22]. These results are best explained by somatic selection for methylation changes in the older mice, changes that give rise to HSC clones with higher rates of proliferation at the expense of effective differentiation.

A strikingly similar observation has been made in the fruit fly, *Drosophila melanogaster*, by Biteau et al. [23]. They showed a decline in the ability of intestinal stem cells (ISCs) to differentiate properly in old (40 days) compared with young (3 days) flies, driven by the JNK gene. This decline in differentiating ability is accompanied by an increased rate

of ISC proliferation.

An interesting, analogous phenomenon has been described by Andrew Wilkie and his colleagues [24, 25], which they call “selfish selection of spermatogonial mutations”. In normal, especially older, males they find clones of spermatogonial cells, effectively the adult tissue stem cells for sperm production, that carry, for example, mutations in the Kras oncogene that are known to cause severe inherited diseases. These mutations appear to be advantageous for the outgrowth of the spermatogonia that carry them but severely disadvantageous to the individuals who inherit these mutations through fertilisation by mutated sperm. They suggest that this form of selfish selection can explain much of the apparent increase in male germ line mutation rates with age.

Origins of somatic genetic variation

Mutation in dividing somatic cells is an unavoidable phenomenon. However efficient the DNA repair processes, every cell when it divides will transmit at least a few new mutations to its daughter cells. There is therefore, as might be expected and as we have already discussed, abundant evidence for the existence of somatic genetic variation in a wide variety of human tissues (see Risques and Kennedy [26] for a recent extensive review). Stable DNA methylation changes, which underlie the whole cellular differentiation process, and which can also be the basis for selected gene expression changes in cancers, are an alternative source of genetic variation that is analogous to mutation. Such methylation changes are likely to be very important functional changes at the somatic level as they occur at a higher rate and can directly influence the level of expression of a relevant gene. Recent evidence suggests rates of gene methylation in HSCs increase with age [22].

While extreme differences in the rates of mutation or methylation due to severe germ line mutations in, for example, certain DNA repair genes will be expected to increase the rate of somatic mutations and methylation changes that we suggest underlie the ageing process, the normal background error rates will, we propose, be enough to lead to normal

rates of ageing. This is entirely analogous to the fact that normal rates of mutation can lead to tumor development, so that genetic instability is not required for tumour initiation [27, 28].

Mutations can either be neutral, meaning they have no functional effect that influences their survival or rate of proliferation, or be selectively disadvantageous leading to a decreased rate of proliferation or survival, or be selectively advantageous resulting in a higher rate of proliferation and/or survival. Cells with neutral mutations can, just by chance, increase in frequency relative to non-mutant cells. While the probability of this is low for any particular mutation, since there is so much of the DNA that can give rise to mutations, clonal variation can arise from neutral genetic variation, but this variation, by its nature, is generally not likely to lead to any relevant functional change. Variants that are disadvantageous to stem cell proliferation will be selected against and so are not expected to play any role in the ageing process. Even quite small selective disadvantages give rise to exceedingly low probabilities of survival. It is therefore only the variants that favor increased stem cell proliferation that are likely to give rise to functionally affected clones. Even quite small selective advantages give hugely increased chances of survival relative to neutral clones (for the classical population genetics analysis behind these arguments see e.g. Cavalli-Sforza and Bodmer [29, 30]).

Mechanisms of somatic selection in adult tissue stem cells

The key to our model for ageing is not just to suggest an effect of selection on the ageing adult tissue stem cells (see, e.g., Beerman et al. [7]), but to propose the underlying basis for the mechanism of action of the selection and the reason for its detrimental effect on adult tissue functions

Thus, we suggest that the common feature in all the examples of age-related functional changes in adult tissue stem cells that we have discussed is somatic selection for mutations (or stable epigenetic changes) that give rise to an increased rate of proliferation and persistence of adult tissue stem cells at the expense of normal functional differentiation and, eventually, apoptosis. It is this process that leads to long term tissue

malfunction. Once an organism has passed the reproductive age, by which is meant the age range during which they have offspring, there is little, if any, counter selection to this inevitable selfish Darwinian process that leads to the gradual erosion of the differentiating capacity of adult tissue stem cells through selection for increased stem cell proliferation. Weaker selective effects, mediated through the benefits that individuals are able to bestow on their younger relatives by helping to care for them [31, 32], may help to explain the relative rarity of tumorigenesis as compared to the normal ageing process during post-reproductive life.

The cancer model of Tomlinson and Bodmer [33] assumes that the overall proliferation rate of stem cells can be represented simply by $\alpha = \alpha_3 - \alpha_2 - \alpha_1$, where α_3 is the rate of cell division, α_2 is the rate at which daughter cells become differentiated and α_1 the rate of apoptosis. In a normal steady state $\alpha = 0$, and so, following this model, if non-mutant stem cells have a negligible apoptosis rate $\alpha_1 = 0$, then $\alpha_3 = \alpha_2$. This can either be achieved by asymmetric cell division, where when a stem cell divides, one daughter always remains a stem cell while the other always becomes differentiated or if, as now seems more likely, stem cells on average produce one differentiated and one stem cell but any given division may produce two daughter stem cells or two differentiated daughter cells. In that case the rate at which stem cells produce two daughter stem cells must equal the rate at which they produce two differentiated cells. The steady state can then be perturbed by a mutation that decreases the rate at which two differentiated cells are produced, so that more daughter stem cells are produced on average than daughter differentiated cells. In that case $\alpha_3 > \alpha_2$ and so $\alpha > 0$ and the stem cells have an increased proliferation rate relative to the steady state maintained by non- mutant stem cells. That is the basis for assuming that mutations which decrease the rate at which a stem cell produces daughter differentiated cells may be the basis for selection for increased rates of proliferation of the stem cells at the expense of normal rates of differentiation. This then disturbs the normal steady state ratio between stem and differentiated cells and so is a basis for tissue malfunction.

Why should these effects be concentrated in adult stem cells, as opposed to differentiated cells? Differentiated cells complete a limited number of divisions before undergoing apoptosis, and they will therefore require a major restructuring of their cellular growth pathways to reactivate a proliferative phenotype. Although this phenotype would confer a selective advantage at the cellular level, it would almost surely not be reached without several mutations. In contrast, in the adult stem cell, one mutation could be sufficient to alter the rate of proliferation versus differentiation.

Differentiated cell populations could, in principle, gain a selective advantage through the acquisition of mutations causing delayed apoptosis, but these mutations could only be passed on to a limited number of daughter cells and would thus have only short-term effects.

Mutations occurring in adult stem cells, *when expressing reduced apoptosis only in their descendant differentiated cells,* would produce a continuing stream of differentiated progeny with longer than normal life spans. These cells would possess an advantage over those undergoing normally programmed cell death, and Tomlinson and Bodmer [33] showed that the longer life spans are not consistent with exponential (i.e. malignant cancerous) growth. Thus, when the apoptosis rate in differentiated cells is small relative to the rate of differentiation, the expected result is a relatively large number of extraneous, differentiated cells, effectively an increasingly large benign tumour. In theory then, this process could be a mechanism for ageing; however, it is unlikely to contribute significantly, as the underlying mutation is not expected to spread beyond a single adult stem cell, or provide any direct selective advantage to the stem cell that carries it. Stem cells will themselves eventually become 'exhausted' and apoptose, and so any descendant population of differentiated cells is likely to be entirely replaced by one undergoing normal programmed cell death. In any case, it seems unlikely that there are common mutations with effects on apoptosis that are large enough to produce sizeable, persistent benign growths emanating from individual stem cell clones.

These arguments against a role for mutations that affect only the function of differentiated cells lead naturally to the suggestion that the predominant cause of ageing lies in the adult stem cells *themselves* evolving a resistance to apoptosis or, more likely initially, to differentiation. This resistance to differentiation, leading to increased proliferation, will result in greater numbers of mutant stem cells, as explained above, and will confer a selective advantage on them. Johnston et al. [34, 35], developing the model of Tomlinson and Bodmer [33] by incorporating homeostatic feedback, showed that successive genetic or stable epigenetically based changes that either decrease apoptosis or decrease differentiation rates in stem cells lead to a series of increasingly large, stable benign tumours, with increasingly large numbers of stem cells and differentiated cells, the latter still having their life spans limited by apoptosis. The incorporation of feedback enables homeostatic regulation of the stem cells and buffering against stochastic physiological variation. This enables the stem cell number to vary around a fixed mean over time, leading to corresponding minor variation in the balance between stem cell division and differentiation. Cancers, resulting from exponential, continuous, growth only arise after the cumulative effect of a number of such steps exceeds the limitations of benign growth. In this sense, the ageing adult tissue stem cells give rise to the analogue of an adenoma rather than a carcinoma. Decreases in the rates of differentiation, and concomitant increases in the rates of stem cell proliferation, that either preserve or do not greatly change the total numbers of cells present in a region of tissue and so maintain basic functionality, will confer smaller selective disadvantages to the organism as a whole than those which cause an overall large growth in numbers and greater disruption of tissue differentiation, as happens in cancers.

As apoptosis occurs infrequently in normally functioning stem cells and is presumed to occur mainly in response to signs of stem cell exhaustion, failure to differentiate properly is very likely to be the candidate for the selective effect of initial mutations. Resistance to apoptosis will confer no significant selective advantage to cells that are not yet displaying

signs of exhaustion. For the *eventual persistence* of the resulting abnormally functioning tissue, there must, however, be further selection specifically against apoptosis of the stem cells carrying the mutations that lead to defective differentiation. This further selection will ensure against full replacement of the overgrown population of abnormally differentiated cells by fully functional, normally differentiated tissue derived from a fresh stem cell, originating from an underlying pool of potential fully functional stem cells. Such replacement will occur in response to signs of exhaustion, including a failure to differentiate at the normal healthy rate. This can explain the presence of, for example, TP53 mutations promoting immortality in ageing adult stem cells, analogous to the presence of TP53 mutations in late larger colorectal adenomas [36]. Failure of stem cell differentiation, associated with persistent increased proliferation, thus acts as a strongly positively selected phenotype at the level of the stem cell, and a weakly negatively selected phenotype at the level of the organism. It is just such changes that we propose are the candidates for many of the causes of normal ageing.

In contrast to the ageing situation, during life up to the end of the reproductive period there is strong selection at the higher level of the individual whole organism, for the integrity of adult tissues over many cycles of stem cell proliferation. As a result, there are mechanisms that differentially protect the adult tissue stem cell from accumulating various forms of damage, including DNA damage, and that ensure the replacement of a stem cell once it has exceeded a certain level of ‘exhaustion’ (for example, see [10]). One feature of this protective process seems to be the existence of two levels of adult tissue stem cells. One level is functional at any given time and is responsible for the clonality of individual colorectal crypts [37], while the second has a slower proliferation rate (some say that cells at this level are quiescent, [38]). The second level 'quiescent' stem cells are poised to replace the first level stem cells as soon as the need arises, namely when a currently fully functional stem cell reaches exhaustion. There is direct evidence for these two levels of stem cells in the human gut [39-42]. It is the counteraction of the exhaustion of individual stem cells, presumably by countering apoptosis, that gives rise to the

persistence of dysregulated differentiated tissue, as discussed above. With this background, we will now discuss in more detail how the model of Johnston et al. [34], with some modification can, we suggest, explain how selection works on adult tissue stem cells.

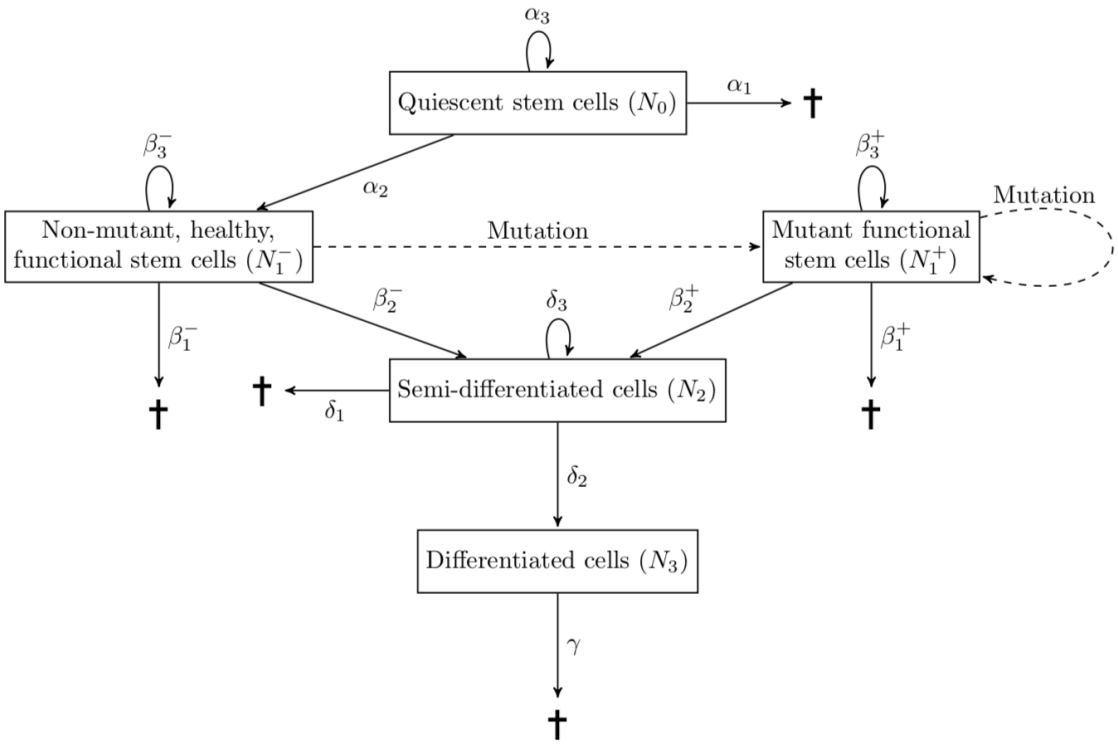
Quantitative model

An extension to the model of Johnston et al. [34], which can explain how long term persistence of individual cells can occur and why this is only likely to happen to cells that have already acquired a failure to differentiate at the full healthy rate, is shown schematically in Figure 1. This shows a graphical depiction of the differentiation process and its relationship to the various parameters. We retain the same general notation as in the original Johnston et al. [34] model, which relates specifically to cell types within the colonic crypt. We represent the number of quiescent or 'second layer' stem cells as N_0, the number of functional stem cells as N_1, the number of semi-differentiated still dividing cells as N_2 and the number of fully differentiated cells as N_3. For each stem cell type we use the parameters α_1, α_2 and α_3 (for quiescent stem cells) and β_1, β_2 and β_3 (for functional stem cells) to represent the apoptosis, differentiation and renewal rates respectively. For semi-differentiated cells we use the equivalent parameters δ_1, δ_2 and δ_3, and for fully differentiated cells we define a single parameter γ, representing their apoptosis rate.

Thus, as depicted in Figure 1, the individual cells of the quiescent stem cell population can, when they divide, die (α_1), become a healthy (non-mutant) functional stem cell (α_2) or self- renew (α_3). Similarly, the healthy functional stem cell can die (β_1), become a transit amplifying semi-differentiated cell, which can self-renew and then become fully differentiated (β_2), or renew (β_3). The same applies to the semi-differentiated cells (δ_1 for dying, δ_2 for differentiating and δ_3 for self-renewal), while differentiated cells can only die by apoptosis (γ).

Figure 1

Schematic representation of a model for stem cell proliferation and differentiation. Crucifix signs indicate cell death.



We assume that when the population of stem or semi-differentiated cells increases, the rate at which new cells are produced also increases, but instead of assuming a linear dependence of per-capita rate on population size we assume that there is a maximum per-capita rate represented by feedback terms in the following equations. This feedback is an essential feature of our model, as it is required to obtain stable states of the relative proportions of the various cell types, where the change in these proportions depends on the magnitude of the effects of mutations on the properties of the functional stem cells. A simple model with no feedback control necessarily results in exponential growth of adult tissue stem cells and so cannot explain what happens with ageing other than giving rise to a cancer. The rates of change of the various cell type counts are as specified by Johnston et al. [34] using the following differential equations:

$$\frac{dN_0}{dt} = \left(\alpha_3 - \alpha_2 - \alpha_1 - \frac{k_0 N_0}{1 + m_0 N_0} \right) N_0 \ , \tag{1}$$

$$\frac{dN_1}{dt} = \left(\beta_3 - \beta_2 - \beta_1 - \frac{k_1 N_1}{1 + m_1 N_1} \right) N_1 + \alpha_2 N_0 + \frac{k_0 N_0^2}{1 + m_0 N_0} \ , \tag{2}$$

$$\frac{dN_2}{dt} = \left(\delta_3 - \delta_2 - \delta_1 - \frac{k_2 N_2}{1 + m_2 N_2} \right) N_2 + \beta_2 N_1 + \frac{k_1 N_1^2}{1 + m_1 N_1} \tag{3}$$

$$\frac{dN_3}{dt} = -\gamma N_3 + \delta_2 N_2 + \frac{k_2 N_2^2}{1 + m_2 N_2} \ . \tag{4}$$

In these equations, the non-linear homeostatic feedback to control cell numbers is captured by the terms $\frac{k_0 N_0^2}{1 + m_0 N_0}$, $\frac{k_1 N_1^2}{1 + m_1 N_1}$ and $\frac{k_2 N_2^2}{1 + m_2 N_2}$. These effectively impose carrying capacities on the three types of cell, which can be overwhelmed when the cells replicate too rapidly. If $m_0 = m_1 = m_2 = 0$ feedback is linear. Cell numbers beyond the limit imposed by feedback are forced to differentiate into the downstream cell type.

Non-linearity is essential to capture the ability of cells to overwhelm homeostatic control, as this is necessary to produce exponential cancerous growths, as shown by Johnston et al. [34].

The model can be expanded to allow for continual replacement of mutant (+) dysregulated stem cells in the functional layer from a supply of 'fresh' non-mutant (-) stem cells produced by the quiescent level of stem cells. We can then show (see Appendix A) that the equilibrium for the total non-quiescent stem cell population size is

$$\tilde{N}_1 = \tilde{N}_1^+ + \tilde{N}_1^- = \frac{\beta^+}{(k_1 - m_1\beta^+)}, \qquad (5)$$

where $\tilde{N}_1^+$ is the mutant stem cell population size, $\tilde{N}_1^-$ is the non-mutant stem cell population size and β^+ the net proliferation rate of the mutant stem cell population. This result, surprisingly, does not depend on any properties specific to the non-mutant functional stem cells.

However, if the mutant survives, this does not cause the elimination of the non-mutant type, as it can also be shown (Appendix A) that when growth is not exponential (namely not cancerous), the healthy non-mutant stem cell type is maintained through continual replenishment from the quiescent stem cells. The numbers of non-mutant and mutant cells at equilibrium (Appendix A) are:

$$\tilde{N}_1^- = \frac{\alpha_2\tilde{N}_0(1+m_0\tilde{N}_0)+k_0\tilde{N}_0^2}{(\beta^+-\beta^-)(1+m_0\tilde{N}_0)}, \qquad (6)$$

$$\tilde{N}_1^+ = \tilde{N}_1 - \tilde{N}_1^- . \qquad (7)$$

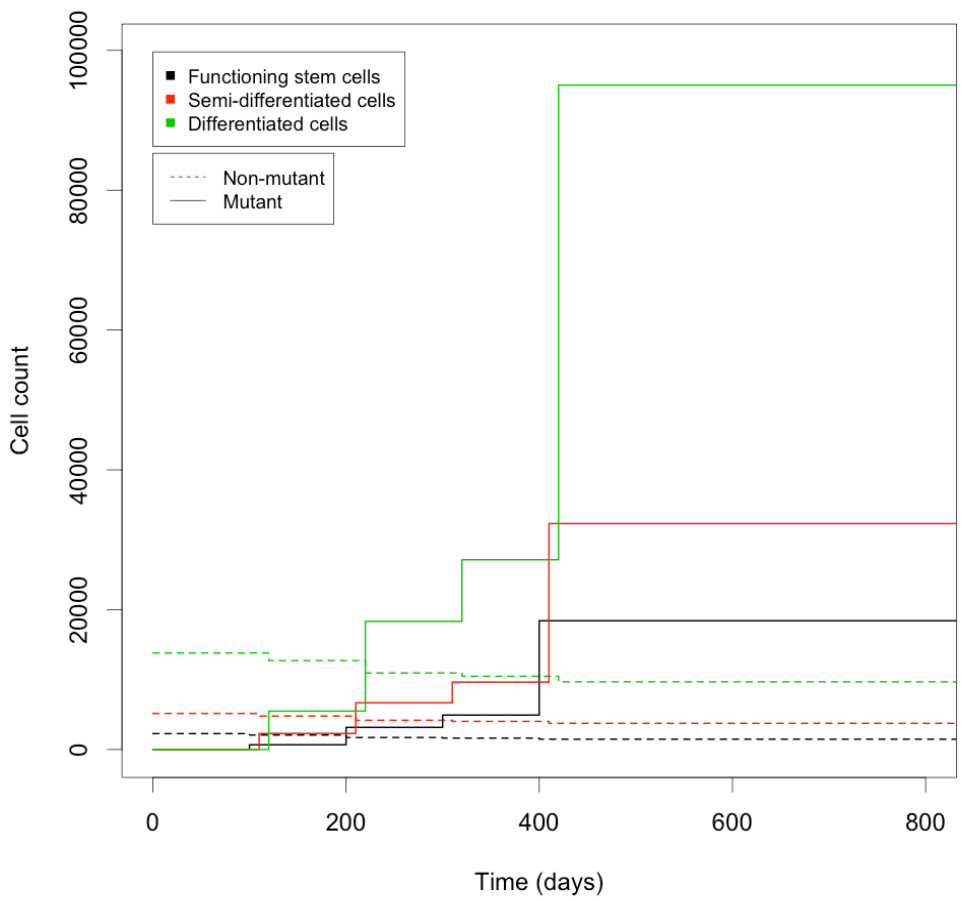
When a new mutant is introduced into a population consisting of an existing mutant type and a healthy non-mutant type, and if it can increase in frequency, the new mutant will entirely replace the original mutant (Appendix A). The new mutation could either be a different mutation in the non-mutant stem cell population (N_1^-) or an additional mutation in the already mutated stem cell population (N_1^+).

The effects of mutations on tissue function will be intrinsic to the mechanisms by which mutations affect differentiation and will also depend on the increased number of resulting differentiated and semi-differentiated cells, N_3 and N_2, which themselves depend on N_1^+, N_1^- and N_0, the numbers of mutant and non-mutant functional stem cells and quiescent stem cells respectively. A numerical example of an outcome of the model based on the

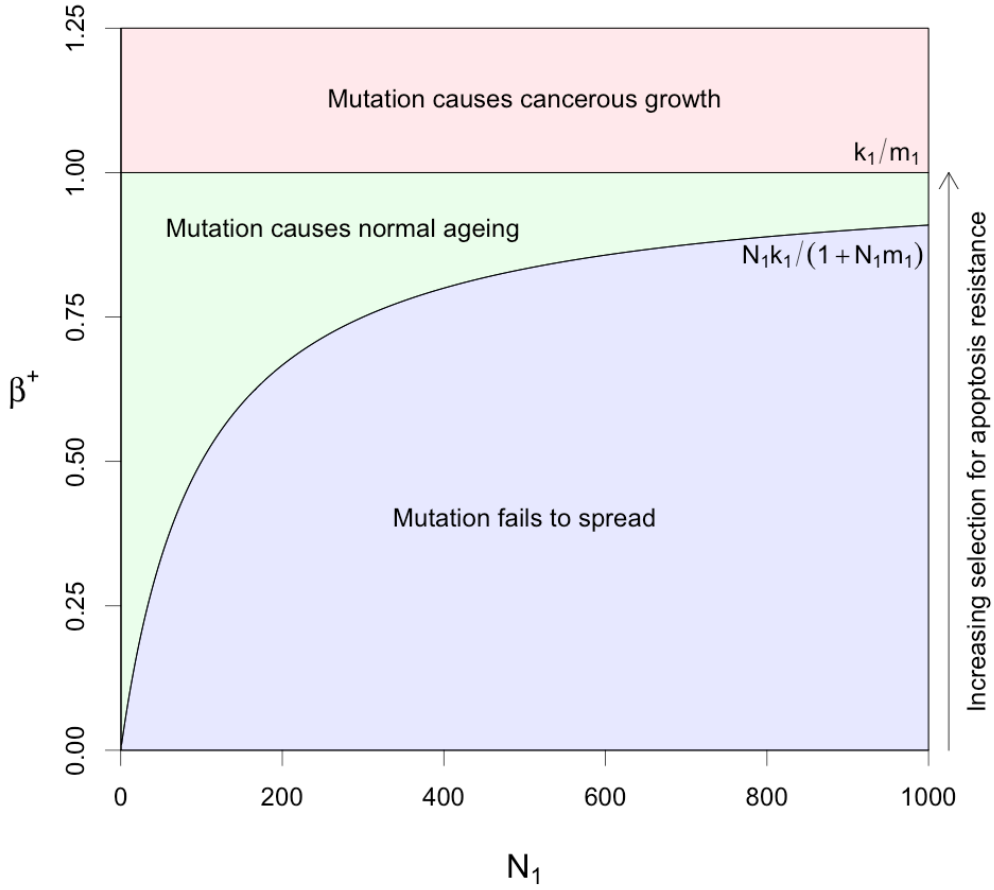
above equations is shown in Figure 2a. This shows equilibrium values for N_1^+ and N_1^- after a series of mutations changing the differentiation (β_2^+) and apoptosis (β_1^+) rate parameters in the functional stem cell population. Consistent with our proposed model, the first three mutations confer a reduced differentiation phenotype, while the fourth and final mutation confers almost complete resistance to apoptosis, in effect immortalisation. The numbers of semi-differentiated and differentiated cells descending from mutant and non-mutant stem cells are also shown. Equilibrium states are calculated from modified equations based on Johnston et al. [35] and are given in Appendix C. The final equilibrium state shows that, now, most of the cells are mutant, with a large number of presumably poorly functioning differentiated cells.

Figure 2

a)An illustrative example of the outcome of the model based on equations 1-4 for a sequence of functional stem cell mutations ($\{\beta_1^+, \beta_2^+\}$= {0.01, 0.1}, {0.01, 0.025}, {0.01, 0.01}, {0.01, 0.005}, and {0, 0.005}) occurring successively every 100 days. The initial values are those for the non-mutant rates, β_1^- = 0.01 and β_2^-= 0.1, which do not vary. Day zero is a point after the end of the organism's reproductive lifespan, when selection for healthy tissue functioning has been greatly diminished. Each vertical line marks a new mutation. Its effect on the numbers of each cell type is given by the horizontal lines, which show stable equilibrium cell counts for each new mutation (See Appendix C). Remaining parameters were set to $k_0 = 0.1$, $m_0 = 0.1$, $\alpha_1 = 0.01$, $\alpha_2 = 0.05$, $\alpha_3 = 1$, $k_1 = 0.01$, $m_1 = 0.01$, $\beta_3^+ = 1$, $\beta_3^- = 1$, $k_2 = 0.001$, $m_2 = 0.001$, $\delta_1 = 0.1$, $\delta_2 = 0.5$, $\delta_3 = 1$ and $\gamma = 0.5$ and did not vary, on the assumption that the mutations only affected the parameters of the functional stem cell population, N_1.



b)Parameter space leading to normal ageing effects (stable growth) and cancerous effects (exponential growth) versus functional stem cell population size. Parameters k_1 and m_1 were both set to 0.01.



Regions of stability in the β parameter space for the system with saturating feedback in the functional stem cell population are shown in Figure 2b, plotted against the functional stem cell population size, N_1. If $\beta^+ < N_1 k_1 / (1 + N_1 m_1)$, then the mutant stem cells cannot sustain their number and the mutant contribution to the tissue region becomes extinct (see Appendix A), resulting in a non-mutant healthy stable tissue region (blue region). If $\beta^+ > k_1 / m_1$ the growth saturation limit is exceeded, and the cell populations grow with no bound as in a cancer [34, 35] (red region). The green region corresponds to normal ageing, where mutant functional stem cells can sustain their growth but do not overwhelm homeostatic non-linear feedback so as to grow exponentially and turn into a cancer.

Discussion

We have shown how adult tissue stem cell growth based on selection, after the end of the reproductive period, for increased stem cell proliferation at the cost of reduced differentiation and eventually elimination of apoptosis, can lead to immortalisation of aberrantly differentiating adult tissue stem cells and stable malfunctioning mature tissue but not cancer. We suggest that this can explain the ageing process and reduced efficacy of the adult tissue stem cells, for those tissues that are continually turning over. This is compatible with observations of increased numbers of somatic clones with mutations that are often found in cancers, as a function of increasing age. In the particular case of HSCs this also fits with the evidence of changes in their patterns of differentiation associated with increasing age, and also associated with oligoclonality of the HSCs.

Our quantitative model is a key part of our case for the role of somatic selection against differentiation and apoptosis in adult tissue stems cells as a major feature of the ageing process. Essential features of the model are that it includes the minimal number of plausible cell compartments needed to model adult tissues, with the minimum necessary number of associated parameters, and that stable states cannot be established without some form of feedback. The aim of such a model is not so much to provide a basis for estimating relevant parameters, such as division and differentiation rates, but to provide a quantitatively and biologically plausible mathematical description of how such selection could work. This is most directly demonstrated by Figure 2b, which shows the limited range of values of β^+, the net functional stem cell reproduction rate, in relation to N_1, the number of functional stem cells, that can lead to functional differentiated tissue without giving rise to a cancer.

The quantitative implementation of the model confirms that failure of functional adult stems cells to differentiate at the normal rate increases their rate of renewal, and so leads generally to stable overgrowths of cells. The model, however, only leads to a cancer when differentiation rates reduce to below a certain threshold. Within growths associated

with mutations, or stable epigenetic expression changes, that increase the net functional stem cell rate of proliferation, β^+, the proportions of the various cell types are different to those observed within healthy tissue, exhibiting an excess of semi-differentiated and differentiated cells. These predictions are entirely consistent with observations of aged tissue.

Clearly for those tissues where there is no apparent turnover connected with tissue stem cells, for example neurons in the brain, ageing may be mostly due to wear and tear with age connected with constant use, just like the parts of a car. However even such tissues are likely to be materially affected by declining function of the stem cells for supporting tissues and cell types, for example, the glial cells in the brain.

On the assumption that, as we have argued, the somatic selection of variant clones with dysregulated differentiation is inevitable and may be a key to human ageing, the basic scientific problem is the lack of understanding of the normal processes of tissue homeostasis before the onset of organismal ageing, and of the changes in these processes with organismal ageing. A systematic study of stem cells and their immediate progeny in a variety of tissues and at different organismal ages, analogous to the study of HSCs by Xie et al. [21], should help to reveal the changes that are associated with ageing. Analyses of mRNA in single cells should reveal the balance of different cell types at different stages of differentiation, while direct DNA and methylation sequencing should reveal the genes in which there are somatic mutations or stable methylation changes in expression in older organisms compared to younger organisms. It will then be interesting to see to what extent the genes in which there are significant changes correspond to the genes in which there are driver mutations or stable methylation changes in cancers from the relevant tissue, as is the case for the somatic mutations found in HSCs from normal healthy elderly individuals [21]. In agreement with this possibility, Slack et al. [43] have suggested that drugs that attack some of the cancer promoting pathways, for example, those associated with mutations in Kras and PTEN, might counter ageing effects. This idea is consistent with the notion that abnormally functioning adult stem cell clones may

accumulate genetic or epigenetic changes by a process of somatic selection for driver mutations or stable methylation changes that is similar to that found in cancers.

Any general 'anti-ageing' treatment must counter successfully all, or at least many, of the Darwinian selection processes acting against normal homeostasis of all the different vital tissues of the ageing human body that are maintained by adult tissue turnover. That is a considerable challenge that may never be fully met. In the meantime, the obvious priority is to continue to try and ameliorate the diseases of old age, including cancer, rheumatoid and osteoarthritis, autoimmune disease and immune function decline, Parkinson's disease, dementias, strokes, heart disease and type 2 diabetes. In this way, we can at least hope to continue to improve the quality of life of the elderly within our existing maximum life span of approximately 100 years.

Conflict of interest

The authors declare no conflicts of interest.

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Appendix

A) Equilibrium cell count and mutant frequency for functional stem cells

The equations for the increase of mutant (N_1^+) and non-mutant (N_1^-) functional stem cell counts are:

$$\frac{dN^+}{dt} = (\beta_3^+ - \beta_2^+ - \beta_1^+)N_1^+ - \frac{k_1 N_1^+ N_1}{1 + m_1 N_1}, \qquad \text{(A1)}$$

$$\frac{dN_1^-}{dt} = (\beta_3^- - \beta_2^- - \beta_1^-)N_1^- - \frac{k_1 N_1^- N_1}{1 + m_1 N_1} + \alpha_2 N_0 + \frac{k_0 N_0^2}{1 + m_0 N_0}, \qquad \text{(A2)}$$

where $N_1 = N_1^+ + N_1^-$, β_1^+, β_2^+ and β_3^+ are the apoptosis, differentiation and proliferation rates in the mutant type and β_1^-, β_2^- and β_3^- are the apoptosis, differentiation and proliferation rates in the non-mutant type. The mutant will increase in number when

$$\beta^+ > \frac{k_1 N_1}{1 + m_1 N_1}, \qquad \text{(A3)}$$

where $\beta^+ = \beta_3^+ - \beta_2^+ - \beta_1^+$, and we also define $\beta^- = \beta_3^- - \beta_2^- - \beta_1^-$. The equilibrium state for N_1^+, setting $dN^+/dt = 0$ in equation (A1) and assuming the mutant increases, is:

$$\tilde{N}_1^+ = \frac{\beta^+}{k_1 - m_1 \beta^+} - \tilde{N}_1^-, \qquad \text{(A4)}$$

where "~" indicates the equilibrium values. The equilibrium value for the total functional stem cell count is therefore

$$\tilde{N}_1 = \tilde{N}_1^+ + \tilde{N}_1^- = \frac{\beta^+}{k_1 - m_1 \beta^+}. \qquad \text{(A5)}$$

Solutions for the numbers of differentiated and semi-differentiated cell types require information on the relative numbers of mutant and non-mutant functional stem cells. The

differential equation for the mutant frequency, $f = N_1^+ / N_1$, is:

$$\frac{df}{dt} = f(1-f)(\beta^+ - \beta^-) - f\frac{\alpha_2 N_0(1+m_0 N_0)+k_0 N_0^2}{N_1(1+m_0 N_0)} \quad , \tag{A6}$$

(Appendix B) which demonstrates an unusual form of natural selection that is weaker when the population size (N_1) is small relative to N_0 and stronger when it is large. When $N_0 = 0$, Equation A6 reduces to a classic population genetic formula stating that the rate of allele frequency change is proportional to $f(1-f)$, the variance in allelic states. Solving $df/dt = 0$ (when $\beta^+ > \beta^-$) gives the equilibrium state

$$\tilde{f} = 1 - \frac{\alpha_2 \tilde{N}_0(1+m_0\tilde{N}_0)+k_0\tilde{N}_0^2}{\tilde{N}_1(\beta^+ - \beta^-)(1+m_0\tilde{N}_0)} = 1-(k_1 - m_1\beta^+)\frac{\alpha_2\tilde{N}_0(1+m_0\tilde{N}_0)+k_0\tilde{N}_0^2}{\beta^+(\beta^+-\beta^-)(1+m_0\tilde{N}_0)} \quad . \tag{A7}$$

When growth is not exponential, namely not cancerous, $k_1 - m_1\beta^+$ is positive, and so the non-mutant type is maintained via continual replenishment by the quiescent stem cells. The number of non-mutant cells is then:

$$\tilde{N}_1^- = \tilde{N}_1(1-\tilde{f}) = \frac{\alpha_2\tilde{N}_0(1+m_0\tilde{N}_0)+k_0\tilde{N}_0^2}{(\beta^+-\beta^-)(1+m_0\tilde{N}_0)} \quad . \tag{A8}$$

When a new mutant is introduced into a population consisting of an existing mutant type and a non-mutant type, the criterion for frequency increase is the same as for the initial mutant (Inequality A3). If increase occurs, the new mutant will entirely replace the original mutant. This is because the differential equation for the proportion of total mutants that are the new mutant type ($f' = N^{++}/(N^+ + N^{++})$ where N^{++} is the number of new mutants), has the same form as the first term of Equation A6,

$$\frac{df'}{dt} = f'(1-f')(\beta^{++} - \beta^+), \tag{A9}$$

where β^{++} is the net growth rate of the new mutant, solving $df'/dt=0$ gives $f=1$ when $(\beta^{++}-\beta^{+})>0$ and $f=0$ when $(\beta^{++}-\beta^{+})<0$. Note that after such a replacement of a previous mutant with a new mutant type, the number of non-mutant functional cells at equilibrium will still be greater than zero (i.e. $\tilde{f}<1$, given by Equation A7) due to their active replacement by quiescent cells.

B: Derivation of the differential equation for mutant allele frequency

The frequency of the mutant is $f=N_1^+/N_1$ where $N_1=N_1^++N_1^-$. Applying the quotient rule gives:

$$\frac{df}{dt}=\frac{dN_1^+}{dt}\frac{1}{N_1}-\frac{dN_1}{dt}\frac{N_1^+}{N_1^2}=\frac{1}{N_1}\left[\frac{dN_1^+}{dt}-\left(\frac{dN_1^+}{dt}+\frac{dN_1^-}{dt}\right)f\right]=\frac{1}{N_1}\left[\frac{dN_1^+}{dt}(1-f)-\frac{dN_1^-}{dt}f\right]. \quad \text{(B1)}$$

Substituting in the derivatives from Equations A1 and A2:

$$\frac{df}{dt}=\frac{1}{N_1}\left[\left(\beta^+N_1^+-\frac{k_1N_1^+N_1}{1+m_1N_1}\right)(1-f)-\left(\beta^-N_1^--\frac{k_1N_1^-N_1}{1+m_1N_1}+R\right)f\right], \quad \text{(B2)}$$

where $R=\alpha_2N_0+\frac{k_0N_0^2}{1+m_0N_0}$. Rearranging using $f=N_1^+/N_1$ and $1-f=N_1^-/N_1$ gives

$$\frac{df}{dt}=f(1-f)\left[\left(\beta^+-\frac{k_1N_1}{1+m_1N_1}\right)-\left(\beta^--\frac{k_1N_1}{1+m_1N_1}\right)\right]-\frac{R}{N_1}f\ , \quad \text{(B3)}$$

and the terms involving k_1 cancel, providing the result in Equation A6:

$$\frac{df}{dt}=f(1-f)(\beta^+-\beta^-)-\frac{R}{N_1}f\ . \quad \text{(B4)}$$

C) Cell counts at equilibrium

The following are adopted from Johnston et al. [34, 35]:

$$\tilde{N}_0 = \frac{\alpha}{k_0 - m_0\alpha} \qquad \text{(C1)}$$

$$\tilde{N}_2 = \frac{1}{2(k_2 - \delta m_2)}\left[\delta + m_2 D_2 + \sqrt{(\delta - m_2 D_2)^2 + 4 D_2 k_2}\right], \qquad \text{(C2)}$$

where $D_2 = [(\beta_3^+ - \beta_1^+)\tilde{f} + (\beta_3^- - \beta_1^-)(1-\tilde{f})]\tilde{N}_1$, the overall differentiation rate in functional stem cells, and

$$\tilde{N}_3 = \frac{\tilde{N}_2}{\gamma}\left(\delta_2 + \frac{k_2 \tilde{N}_2}{1 + m_2 \tilde{N}_2}\right), \qquad \text{(C3)}$$

where $\alpha = \alpha_3 - \alpha_2 - \alpha_1$ and $\delta = \delta_3 - \delta_2 - \delta_1$. The number of semi-differentiated cells descending from mutant functional stem cells ($\tilde{N}_2^+$), shown in Figure 2a, is given by Equation C2 replacing D_2 with $D_2^+ = (\beta_3^+ - \beta_1^+)\tilde{N}_1^+$ when $f > 0$, and $\tilde{N}_2^+ = 0$ when $f = 0$. The number descending from non-mutant functional stem cells ($\tilde{N}_2^-$) is obtained by replacing D_2 with $D_2^- = (\beta_3^- - \beta_1^-)\tilde{N}_1^-$. Likewise, the numbers of differentiated cells descending from mutant ($\tilde{N}_3^+$) and non-mutant ($\tilde{N}_3^-$) functional cells are given by Equation C3 after replacing $\tilde{N}_2$ with $\tilde{N}_2^+$ and $\tilde{N}_2^-$ respectively.

The equilibrium state for N_1 depends on whether there are mutant cells present. When there are no mutant cells at equilibrium, i.e. when all functional stem cells are genetically identical to their ancestral quiescent layer cells, the equilibrium for N_1 is, from Johnston et al. [34, 35],

$$\tilde{N}_1 = \frac{1}{2(k_1 - \beta^- m_1)}\left[\beta^- + m_1 D_1 + \sqrt{(\beta^- - m_1 D_1)^2 + 4 D_1 k_1}\right], \qquad \text{(C4)}$$

where $D_1 = (\alpha_3 - \alpha_1)\tilde{N}_0$, the quiescent cell differentiation rate. When there are mutants

that can grow fast enough to reach a stable equilibrium (Inequality A3), the stable numbers of non-mutant and mutant functional stem cells are given by Equations A4 and A8 and Equations 6 and 7 in the main text.

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