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Stem cells, ageing and the quest for immortality

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Adult stem cells reside in most mammalian tissues, but the extent to which they contribute to normal homeostasis and repair varies widely. There is an overall decline in tissue regenerative potential with age, and the question arises as to whether this is due to the intrinsic ageing of stem cells or, rather, to the impairment of stem-cell function in the aged tissue environment. Unravelling these distinct contributions to the aged phenotype will be critical to the success of any therapeutic application of stem cells in the emerging field of regenerative medicine with respect to tissue injury, degenerative diseases or normal functional declines that accompany ageing.

The woods decay, the woods decay and fall,
The vapours weep their burthen to the ground,
Man comes and tills the field and lies beneath,
And after many a summer dies the swan.
Me only cruel immortality
Consumes;

from *Tithonus*, by Alfred Tennyson

Stem cells have captured the popular imagination with the promise of enhanced tissue repair, the treatment of degenerative diseases and even the amelioration of dysfunction associated with normal ageing. The possibility that stem cells could have therapeutic applications in the context of normal ageing raises two related questions. First, what is the effect of ageing on stem cells themselves? Second, to what extent can the functional decline of a tissue be attributed to a limited (and perhaps declining) capacity of the resident stem cells to maintain normal tissue structure and function?

The first question has been amenable to study in specific cases. As a starting point, one needs to identify and isolate stem cells, assess their numbers and functionality, and then apply those assays across the lifespan to test for changes with age. The real challenge is then to try to understand the causes and mechanisms of those changes. The second question is much more difficult to address experimentally because of the tight link between stem cells and the tissues in which they reside. As such, there are no tissues for which this question has been definitely answered. Furthermore, the issue is vastly different depending on whether the tissue exhibits high cellular turnover, low cellular turnover but high regenerative potential in response to damage, or low cellular turnover and negligible regenerative potential (Fig. 1). The role of stem cells in the biology of these different classes of tissues is so distinct that the extent to which ageing of the stem-cell compartment might affect the tissue cannot be extrapolated from one tissue to another. The focus of this review is the intersection between the biology of ageing and the biology of adult stem cells, paying particular attention to mammalian tissue biology, functional changes with age in those tissues that might or might not relate to stem cells, and the relationship between tissue ageing and longevity.

Ageing, evolution and stem-cell immortality

Evolutionary theories of ageing

The study of how stem cells change with age leads inevitably to the underlying question, which can only be couched in evolutionary terms, of why we age. The value of asking this question is not to unravel a certain purpose of ageing, but rather to frame the processes of ageing within evolutionary constraints that might have been imposed by

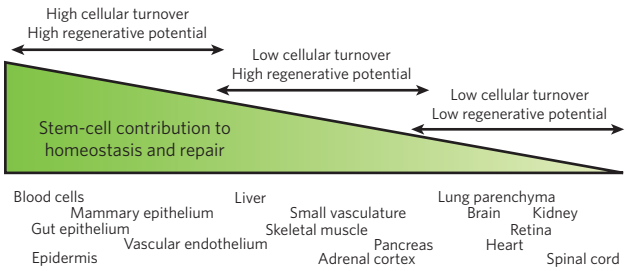

Figure 1 | Tissue heterogeneity and stem-cell functionality for homeostasis and repair. The extent to which the effects of ageing on the resident stem cells determine the phenotype of an aged tissue is likely to correlate with the extent to which stem cells are responsible for normal tissue homeostasis and repair. Along this spectrum, tissues generally fall into one of three categories. First, tissues with high turnover (such as blood, skin and gut) have a prominent stem-cell compartment and, by definition, have high regenerative capacity. Second, tissues with low turnover but high regenerative potential might use different strategies to ensure effective repair in the setting of acute injury. In skeletal muscle, for example, differentiated myofibres are unable to proliferate to generate new tissue, so muscle must rely on resident stem cells for all turnover and repair[63]. For the liver, it seems that differentiated hepatocytes can proliferate sufficiently to mediate effective tissue remodelling, repair and replacement normally[64], whereas stem cells might be recruited in the setting of severe injury[65]. Third, tissues with low turnover and low regenerative potential might have stem cells that mediate only limited tissue repair. Although there is much interest in harnessing the potential of stem cells in the brain[66] and heart[67] for therapeutic purposes, for example, there is limited endogenous repair capacity of these tissues following acute injuries.

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selective pressures. Evolutionary theorists have entertained the possibility that ageing is genetically programmed, based on the notion that elimination of individuals who are past their reproductive prime would be beneficial for the species, as this would theoretically preserve resources for the most reproductively fit individuals and for future generations. However, this view has not withstood rigorous analysis, primarily because the 'aged phenotype' is rare in nature[1]. The majority of animals in the wild succumb to starvation, predation, exposure, disease or accident long before the appearance of characteristics that are recognized as ageing in humans and in domesticated and laboratory animals.

Two theories have emerged that integrate the possibility of genetic programmes that direct the ageing process with the notion that such programmes are not likely to have arisen by pressures of natural selection[2]. The first is the 'mutation accumulation' theory, first proposed by Medawar[3], which posits that mutations leading to detrimental age-related changes could accumulate over successive generations if their effects were only realized well after the age of peak reproductive success. As few individuals would actually reach those ages, such mutations would escape negative selective pressure. The second theory, the 'antagonistic pleiotropy' theory[4], postulates that there might be genes whose expression is harmful in later life that accumulate in populations not because they are silent earlier in life but because they are actually beneficial to survival or reproductive fitness. Such mutations could thus have a selective advantage. Although these concepts have guided attempts to merge evolutionary theory with empirical studies of the biology of ageing, little evidence of cumulative mutations, and only rare examples of genes that display antagonistic pleiotropy, have been found[2,5].

Nevertheless, there is overwhelming evidence that there are strong genetic influences on the rate of ageing. Perhaps the most compelling evidence is that the differences of rates of ageing within individuals of a species are negligible compared with the vast differences across species[6]. A mayfly (a member of the aptly named order Ephemeroptera) moults, reproduces and dies within a single day, in some cases with a functional lifespan measured in hours; by contrast, giant tortoises can live for almost 200 years (Fig. 2). The powerful influence of genetics is further reflected by the ever increasing number of single-gene mutations that can influence the lifespan of eukaryotes ranging from yeast to mice[7–13].

A theory that provides a more generalizable framework for considering the influences of genetics on the ageing process is the 'disposable soma' theory[14]. A key aspect of this theory is the distinction between the immortal germ line and the 'disposable' (that is, mortal) soma. The basic premise is that species have evolved with genetic programmes that optimize the utilization of resources for survival and reproduction. Ageing occurs through 'wear and tear', and the genetic programme of any species is designed to resist that damage long enough for organisms to reach reproductive maturity. Any increased environmental stress that challenged the soma would divert limited resources away from reproduction. This is consistent with the well recognized, although not obligatory, negative correlation between lifespan and fecundity[15]. This theory also provides a framework for integrating the effects of caloric restriction on prolonging lifespan and reducing fecundity[15], and the intriguing fact that so many of the single-gene mutations that prolong lifespan are involved in pathways of energy metabolism and stress responses[8,9,12,13,16].

The disposable soma theory also provides an interesting, if unanticipated, framework for considering adult stem cells in relation to both the disposable soma in which they reside and the immortal germ line. Are adult stem cells more similar to the rest of the soma or to the cells of the germ line?

Box 1 | Self-renewal: a misnomer and an impossible standard to meet

One characteristic that is demanded of a cell to be admitted into the stem-cell pantheon is the ability to self-renew. Is this fair? It depends on how rigorously applied the definition of self-renewal is. Clearly, no cell could meet the standard if self-renewal were defined as cell division without any errors of DNA replication. Yet, if such subtle molecular differences between mother and daughter cells are allowable within the definition of self-renewal, what other differences are also allowable? Clearly, for practical purposes, the issue of self-renewal is a physiological one. Does the daughter cell possess two key functional features of the mother cell, namely, the ability to generate the same spectrum of differentiated progeny and the ability to generate another daughter cell that can also generate the same spectrum of differentiated progeny? However, just as too rigorous a molecular definition renders self-renewal impossible to achieve, even this looser physiological definition requires that the criteria are not applied too stringently. For example, is it a failure of self-renewal if the daughter cell can generate the same types of differentiated progeny as the parent but fewer of them, or if the daughter cell's progeny, when induced to differentiate, are skewed towards one particular differentiated phenotype?

Within the context of ageing, this gradual change of genotype and phenotype across generations, or 'replicative ageing', is superimposed upon the chronological ageing that occurs in any cell with the passage of time (Fig. 5) — a distinction that has been elegantly made in ageing studies of yeast[62]. In either replicative or chronological ageing, genetic drift through mutation accumulation governs intrinsic stem-cell ageing and affects stem-cell functionality. These kinds of changes directly pertain to two levels of analysis that are commonly used to characterize stem cells: the pattern of expression of proteins by flow cytometry or immunocytochemistry, and the pattern of transcript expression by microarray analysis. The important issue is not whether the patterns detected in the progeny differ from the patterns detected in the parental population, but rather whether there is any functional loss. The renewal of function supersedes any definition of self-renewal at a molecular level. This is not to discount the value of markers for identifying stem cells and more committed progeny; rather, it is a caution against having too stringent criteria for self-renewal, particularly with regard to molecular determinants.

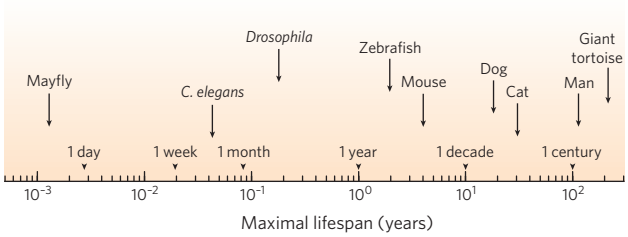

Figure 2 | Variation of maximal lifespan across species. Presented on a logarithmic timescale, this figure illustrates the vast range of maximal lifespans at the extremes (mayfly and giant tortoise), in some of man's favourite pets (dog and cat) and experimental animals (*Caenorhabditis elegans*, *Drosophila*, zebrafish and mouse), and in man himself.

The immortal germ line and the mortal soma

Although the distinction between the mortal soma and the immortal germ line is unambiguous in mammalian species, it is less clear across a broader phylogenetic spectrum. Seemingly unlimited regenerative capacity of appendages and limbs is seen in organisms such as salamanders and starfish[17,18]. This regeneration of body parts does not produce new individuals in these species, but it does begin to hint at the possibility. It is in organisms such as planaria and hydra that 'somatic' parts are capable of giving rise to entirely new individuals[19,20], which is more reminiscent of the immortality of the germ line or of single-celled organisms that divide by fission.

The concept of immortality differs when considering the germ line, the early cells of the embryo that can be derived as embryonic stem (ES) cells and studied *in vitro*, and the soma. For the germ line, immortality is dependent on adaptive change and natural selection promoting survival and reproductive success associated with advantageous

changes in the genome, and is gauged over evolutionary time. For ES cells, the criteria for immortality are determined by the investigator. Progeny with changes that would confer a selective growth advantage are deemed unfavourable and discarded because they are usually the result of karyotypic changes[21]. Thus, immortality of stem-cell lines is assured by selecting progeny with properties as close to the parental cells as possible, not by the natural selection of competitive reproductive advantage. Still, genetic drift will inevitably occur over time and immortality of the line might be measured in decades, not eons. For the soma, the conventional meaning of immortality is 'living forever', which speaks more of mythology than of science. Nevertheless, the prospect of increasingly long-lived individuals emerges from scientific study of lifespan and its extension. One is, however, reminded of the Greek myth of Tithonus, a mortal who became the lover of the goddess Eos. When Zeus stole away another of Eos's mortal lovers, he promised to fulfil one wish to repay her. Eos asked Zeus to grant Tithonus immortality. Unfortunately, she forgot to include eternal youthfulness in her wish. Oops. As a result, although Tithonus was given the 'gift' of immortality, he continued to age, withered beyond recognition and ended up begging for death for all of eternity.

A feature of germline immortality that is important for adult stem cells is the ability to ensure that genetic information is passed on with the highest fidelity to successive generations. Cells must have robust mechanisms to resist and/or repair damage to the genome[22]. For ES cells, the maintenance of genome stability is essential to their value as tools for research and potential therapeutic vehicles[23]. For adult stem cells *in vivo*, possessing the capacity to resist, detect and repair changes in the genome (such as telomere shortening and mutation accumulation) underlies their ability to participate in tissue homeostasis and repair across an organism's lifespan. Fundamentally, this is at the heart of one of the key features that defines stem cells, namely, the ability to self-renew, although the extent to which maintaining genomic integrity is imperfect means that the criteria for self-renewal must not be too stringent (Box 1). Finally, the possibility that many forms of cancer might be the result of acquired mutations in adult stem cells highlights an even more important consequence, at least in terms of health, of a failure to maintain genomic stability in adult stem cells[24].

The ageing process and adult stem-cell functionality

The hierarchical nature of biological defences

The process of organismal ageing is characterized by functional decline due to histologic and biochemical changes in tissues and organ systems with the passage of time. Declining functionality is paralleled by diminishing capacity to respond to injury or stress. As stem cells are involved in homeostasis as well as regeneration and repair for many tissues, the question naturally arises as to whether the characteristics of an ageing tissue can be understood as a declining functionality of the adult stem cells that reside within it. However, this question would seem to place stem cells in a unique position as guardians of tissue youthfulness, rather than within the hierarchy of homeostatic mechanisms that decline with age, ranging from the molecular to the organismal (Table 1). The accumulation of mutations in nuclear and mitochondrial DNA, despite the range of repair mechanisms for preventing such accumulation[25], sits at the pinnacle of the hierarchy, representing the most fundamental and irreversible changes from which many others follow. Stem cells contribute at an intermediate level of tissue homeostasis and repair, and, as noted above (Fig. 1), might contribute negligibly to the ageing phenotype for tissues with extremely low cellular turnover. In the heart and brain, for example, where the overwhelming majority of cardiomyocytes and neurons, respectively, are not replaced during adult life, the aged phenotype is best understood in terms of changes in these postmitotic cells rather than any stem-cell compartment.

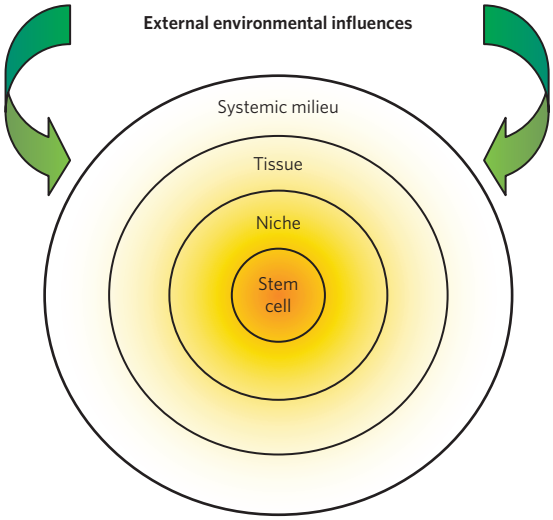

Figure 3 | Influences on stem-cell functionality. Adult stem cells interact dynamically with their environment at increasing levels of complexity. Locally within their niche, stem cells respond to the extracellular matrix, to other cells within the niche and to paracrine factors. Within the tissue, the stem cell/niche unit is influenced by soluble factors derived from parenchymal cells, which are, in turn, influenced by systemic immunological and neuroendocrine signals. Finally, external environmental influences, which are particularly relevant to discussions of ageing, filter down through these various levels to influence stem-cell function.

Table 1 | Manifestations of ageing and homeostatic defences

Position in hierarchy	Manifestations of ageing	Homeostatic mechanisms/defence
Molecular changes that lead to cellular dysfunction	Cumulative mutations in nuclear and mitochondrial DNA	DNA repair activities; telomerase activity
	Oxidative damage to cellular constituents	Antioxidant enzymes, cytosolic and membrane free-radical scavengers
	Accumulation and aggregation of abnormal proteins, lipids and other macromolecular constituents	Mechanisms to recognize and degrade abnormal proteins and other macromolecules
Cellular changes that lead to tissue dysfunction	Cell death	Anti-apoptotic pathways
	Oncogenesis	Cell-cycle checkpoints, tumour-suppressor genes, apoptosis pathways
	Senescence	Immune surveillance
Tissue changes that lead to organismal dysfunction	Atrophy from cell loss and diminished regenerative capacity	Stem cells for tissue maintenance and repair
	Extracellular matrix changes	Matrix remodelling activities such as those of metalloproteinases
	Extracellular deposits	Phagocytic activities of resident and circulating cells

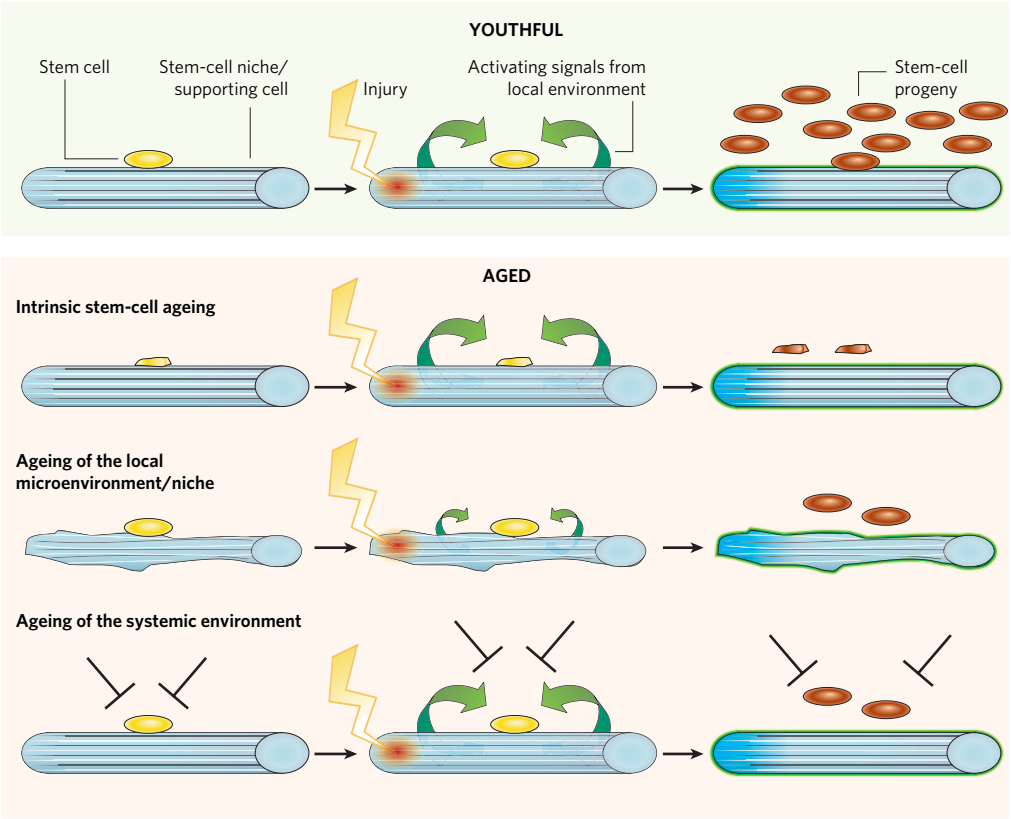

Figure 4 | Ageing of stem-cell functionality. The decline in stem-cell functionality with age can be due to age-related changes at many levels. This figure illustrates several possibilities using a skeletal muscle fibre and an associated stem (satellite) cell as a model. In response to tissue injury, local signals induce satellite cells to begin proliferating in order to generate sufficient progeny for tissue repair. Age-related changes in satellite cells, in the satellite-cell niche or in the systemic milieu could all result in a diminished functionality of satellite cells in an aged organism, manifested as a decreased propensity to generate sufficient functional progeny for effective regeneration.

Stem-cell functionality and the stem-cell niche

Almost every tissue studied has shown age-related decrements in the rate and/or efficacy of normal cellular turnover and regeneration in response to injury. Whereas this strongly suggests an age-related decline in stem-cell functionality, it does not necessarily imply that there is intrinsic stem-cell ageing. Stem-cell function is regulated at increasing levels of complexity, from cell-autonomous regulation to regulation by the local environment of the stem-cell niche, the surrounding tissue, the systemic milieu of the organism and, ultimately, the external environment (Fig. 3). Furthermore, there are interactions among these compartments such that the systemic milieu is the product of all the various tissue/niche/stem-cell units throughout the organism. Declining tissue homeostasis or repair could arise from age-related changes in the numbers or properties of stem cells, in the local environment or niche in which the stem cells reside, in the systemic milieu of the organism that influences all cells, or in any combination of these. Changes within the niche would include alterations in the amount and composition of the extracellular matrix, changes in the membrane proteins and lipids in cells that make direct contact with stem cells, and changes in soluble paracrine and endocrine factors that constitute the systemic milieu. Studies of the importance of such changes in the stem-cell niche have been best described in the germline stem-cell niche in *Drosophila*[26,27]. Systemic changes would include immunological and neuroendocrine changes, and, in the case of tissue injury or disease, changes in the factors that are released from damaged cells and in the inflammatory response that accompanies such damage. These effects have been demonstrated in heterochronic transplantation or parabiosis studies in which cells from aged animals are exposed to systemic factors of young animals (and *vice versa*)[28–31]. Thus, even in the absence of significant ageing of stem cells themselves, stem-cell functionality could show a marked age-related decline due to decrements in the signals within the local and systemic environment that modulate the function of stem cells or their progeny (Fig. 4).

Stem cells and longevity

Even more enigmatic than the role of stem cells in tissue ageing is the relationship between stem cells and longevity itself. There is no evidence that the lifespan of any species is determined by a limited supply or limited functionality of its stem-cell populations, and yet this association is commonly made[32,33]. There is a risk of tautologies if one starts with the premises that declining stem-cell function is responsible for tissue ageing and that tissue ageing determines longevity. A major weakness of the first premise is the negligible role of stem cells in the ageing of tissues with low cellular turnover. The second premise fails to acknowledge the experimental and conceptual gap that exists between our understanding of tissue ageing and the determinants of lifespan. Common sense dictates that there must be a relationship between the two, and experimental interventions that alter the lifespan of model organisms also tend to alter tissue ageing, but the direct link remains elusive. It is clear that single-gene mutations that extend maximal lifespan also result in structural and physiological changes in tissues that indicate a slowing of the ageing process and a reduction in age-related pathologies[12,13]. Likewise, the increased longevity from caloric restriction in various species is accompanied by an apparent reduction in the rate of tissue ageing[34–37]. However, none of these important advances in the biology of ageing provide any direct evidence as to why aged individuals within a species die in the absence of identifiable injury or disease. The cause of death from 'old age' remains one of the central mysteries of biology (Box 2). However, there is no evidence that the maximal lifespan of any species is determined by declining stem-cell function or, conversely, that increasing the number or functionality of any single stem-cell population would extend lifespan. One need only look at species with tissues that are almost entirely postmitotic to see that there cannot be any tight mechanistic link between adult stem-cell function and longevity in any evolutionary sense. Longevity *per se* is several steps removed from the biology of stem-cell ageing, linked primarily by how genetic variations and environmental factors might influence each, perhaps coordinately, but not by any demonstrated causal relationship.

Tests of intrinsic ageing of adult stem cells

Given the inherent complexity of distinguishing intrinsic cellular ageing from ageing of the cellular milieu when stem cells are studied in their native environment, the most direct tests of intrinsic cellular

ageing have been those that have involved isolation of stem cells from young and old animals, and transfer to identical *in vitro* or *in vivo* environments. This has been done extensively for only a few stem-cell populations. An important distinction even using these more rigorous assays of stem-cell ageing is whether any cell-autonomous changes that are detected are stable and hereditable, or if they are reversible. In some cases, stem cells from aged animals show a delay in responsiveness to activating stimuli, ultimately yielding comparable results to those obtained from young stem cells, suggesting that the initial responses might reflect epigenetic modifications rather than irreversible (at least under physiological conditions) genetic or biochemical changes. Generally, the effects of age on isolated stems cells are compared in assays of growth, differentiation, apoptosis, transformation and senescence. Although cellular senescence has been characterized primarily as an *in vitro* phenomenon related to replicative lifespan[38,39], it has also been proposed that cellular senescence occurs *in vivo* and might result in a toxic 'gain of function' by promoting an environment that fosters cellular transformation[40].

Two specific examples of adult stem cells — haematopoietic stem (HS) cells and skeletal muscle satellite cells — are highlighted below because they have been prospectively isolated, and the effects of ageing have been studied both *in vivo* using transplantation assays and *in vitro*. In addition, although both populations are derived from tissues with high regenerative potential, they also represent opposite ends of the spectrum of tissues in terms of normal cellular turnover (Fig. 1). As such, the stem cells and their progeny in blood will be much more affected by the combined effects of replicative and chronological ageing than will the stem cells and their progeny in skeletal muscle, which are likely to experience primarily chronological ageing (Fig. 5).

Haematopoietic stem cells

These give rise to the cellular constituents of blood that serve life-sustaining functions, such as oxygen transport, blood coagulation and immune function. With age, there is evidence of a gradual decline in all of these functions[41,42]. Such changes are not due to the gradual depletion of HS cells, as there is actually an increase, rather than a decrease, in HS cells with age[43]. *In vitro* studies of isolated HS cells have shown that there is no difference between young and old cells in terms of their ability to form colonies and their proliferative potential[44]. Likewise, there does not seem to be any decline with age of the ability of HS cells to interact with stroma *in vitro*[44].

When HS cells have been tested in serial-transplantation experiments, complete reconstitution of the blood occurs over several lifespans in mice[45], and old HS cells are as effective as young HS cells at reconstituting the blood lineages after transplantation[28,46,47]. In addition, the size of individual stem-cell clones in recipients receiving single competitive-repopulation units is independent of age[48]. However, aged HS cells seem to be less effective at homing and engrafting[44], suggesting that intrinsic ageing of HS cells can be revealed by this type of analysis. The extent of intrinsic ageing of HS cells also seems to be strain dependent, as determined by competitive-repopulation studies[46,49]. These cell-intrinsic changes might be epigenetic, as they could be reversed when the cells were placed in the appropriate environment[50]. In these assays, cells of different genetic backgrounds responded differently, highlighting the importance of genetic influences on the ageing process. Recently, direct molecular analysis of purified HS cells revealed differences in gene expression between cells from young and old mice[51]. It is intriguing that the changes in specific genes were correlated with the noted skewing of the lineage potential of aged HS cells[52]. It will be interesting to test whether these cell-intrinsic changes are also reversible and dependent on the environment in which the cells reside.

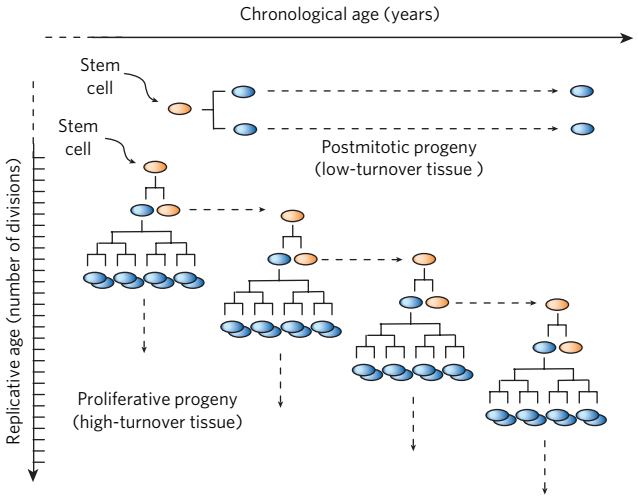

Figure 5 | Chronological and replicative ageing. Attempts to determine the age of a cell are confounded by the distinction between chronological and replicative ageing. Certain stem cells may give rise to postmitotic cells that are present in the tissue for the entire lifespan of the organism. Such postmitotic cells experience primarily chronological ageing. To the extent that the stem cells within those tissues remain quiescent, they would also experience mainly the effects of chronological ageing. By contrast, certain stem cells, particularly those in tissues with high turnover, divide regularly and generate progeny that undergo extensive proliferative expansion. There is an additional component of ageing for those stem cells and their progeny — that is, replicative ageing. On the basis of the evidence that, depending on the species, mammalian somatic cells have a limit to their replicative potential related to genomic instability from telomere shortening, mutation accumulation and genomic rearrangements[68], proliferative cells will integrate the effects of chronological ageing with the effects of replicative ageing.

Skeletal muscle satellite cells

The primary skeletal muscle stem cell (the satellite cell) is quiescent in adult muscle, but is activated to proliferate and generate committed progeny in response to injury or disease[53]. Estimates of satellite cell number with age vary depending upon the method used, ranging from a slight increase[54] as reported for HS cells, to a minimal change[55] or a decline[54,56]. However, there is a much greater impairment of regenerative potential with age than can be accounted for by any decline in number[57].

Box 2 | Dying of old age

There is no compelling explanation for the cause of death in old but otherwise healthy humans, mice, worms or flies, or any other organism for that matter. The colloquial expression 'dying of old age' belies our knowledge of the biological basis of this event. Surely, the cessation of respiratory and circulatory functions results quickly in irreversible damage to vital organs; however, to insist that ageing of the heart or lungs is the cause of death only sidesteps the question. Examination of tissues of an old member of a species at the time of death will reveal stereotypical biological changes and perhaps even pathological changes that were only mildly symptomatic or even asymptomatic. Why, then, did this individual die? We can measure average and maximal lifespan in species, we can evaluate the effects of genetic, nutritional or pharmacological interventions that alter those indices, and we can correlate them with changes in tissue ageing. Yet no hypothesis has emerged that yields a useful definition of dying of old age in terms of cell and tissue biology. In the absence of an acute event or an overwhelming disease, the ageing process affects all tissues and cells, whether they are postmitotic, actively mitotic or quiescent. The result is a recognizably progressive change for which the actuarial definition of increased probability of dying corresponds to empirical biological and physiological changes, but fails to provide any clue as to the mechanism. The question 'When does life begin?' engenders much debate, and the biological mechanisms that underlie the processes suggested as answers — for example, fertilization, gastrulation and implantation — are intensely studied. Although much effort has gone into defining death from a medical and legal perspective, little effort has been devoted to studying the processes that lead to 'death from old age', and rarely is the question asked from a biological perspective, 'When does death begin?'

This age-related decline of regenerative potential has been attributed to impairment in aged muscle of the Notch signalling pathway, which is essential for normal satellite-cell activation in young animals[55].

Initial *in vitro* studies of satellite cells from young and old animals suggested that there was intrinsic ageing of this stem-cell population, as aged cells generated far fewer progeny[58,59]. However, this interpretation was at odds with the finding that regeneration mediated by aged satellite cells was highly effective when the cells were transplanted into young animals as whole-muscle grafts[30]. In fact, the results were indistinguishable from the regeneration mediated by grafting of young muscle. These data suggest reversible epigenetic modifications of aged muscle stem cells, an interpretation supported by recent data showing that aged muscle stem cells, when exposed to a youthful systemic milieu by virtue of parabiotic pairings of aged and young mice, activate and repair muscle nearly as well as young satellite cells[31]. Thus, it seems that the diminished regenerative potential of aged muscle is not primarily due to intrinsic ageing of satellite cells, but rather to the effects of the aged environment on satellite-cell function. Gene-expression studies of cells derived from satellite cells have shown changes with age[60]. However, as with HS cells, it is not clear whether these transcriptional profiles are due to irreversible genetic changes or reversible epigenetic effects.

Fantasies and realities concerning stem-cell therapeutics

The area of health and ageing that is likely to benefit soonest from advances in the biology of adult stem cells is the emerging field of regenerative medicine. This could involve either the enhancement of endogenous stem cells or the transplantation of exogenous stem cells that have been expanded in culture. Transplanting exogenous stem cells into damaged tissues will be firmly founded on the principles of transplantation biology and immunology. However, an appreciation of the importance of the niche, especially the aged niche, will be critical to the success of stem-cell transplantation in enhancing tissue repair in the setting of acute injury. The importance of the host environment becomes even more complex when considering the use of stem cells in the treatment of chronic degenerative diseases.

Despite the historical quests for immortality and continuing interest in extending human longevity[61], the application of stem-cell 'therapeutics' to delay the ageing process itself is even more remote. Given the universality of the ageing process and the profound influence of the systemic milieu on cells within a tissue, the theoretical basis of such an application is unclear. Any translation from animals to humans of experimental methods for increasing longevity is more likely to emerge from an understanding of the systemic coordination and regulation of cellular metabolic activity and cellular defences, although any extension of lifespan is sure to come at some cost.

Nevertheless, the potential for stem cells to be used as therapeutic vehicles has had a profound effect on the vision of the future of regenerative medicine. The suppression of adult stem-cell proliferation by the systemic milieu in aged animals, although it limits tissue regenerative potential and possibly promotes senescence or apoptosis, might be a crucial defence against the development of cancer, the likelihood of which increases with the accumulation of mutations in the stem-cell genome with age. Therapeutic applications of adult stem cells to aged tissue repair in the context of regenerative medicine will require an increased understanding of stem-cell biology, the environment of the aged tissue and the interaction between the two. ■

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