STEM CELLS

How stem cells age and why this makes us grow old

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Abstract | Recent data suggest that we age, in part, because our self-renewing stem cells grow old as a result of heritable intrinsic events, such as DNA damage, as well as extrinsic forces, such as changes in their supporting niches. Mechanisms that suppress the development of cancer, such as senescence and apoptosis, which rely on telomere shortening and the activities of p53 and $p16^{INK4a}$, may also induce an unwanted consequence: a decline in the replicative function of certain stem-cell types with advancing age. This decreased regenerative capacity appears to contribute to some aspects of mammalian ageing, with new findings pointing to a 'stem-cell hypothesis' for human age-associated conditions such as frailty, atherosclerosis and type 2 diabetes.

Forkhead transcription factor class O
(FOXO). One of a family of evolutionarily conserved transcription factors that are linked to lifespan regulation in lower systems and to stem-cell maintenance in mice. The FOXO proteins are thought to exert these effects by regulating the expression of genes involved in apoptosis, proliferation, glucose metabolism, DNA repair, regulation of reactive oxygen species and other diverse cellular processes.

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Diverse acquired and genetic factors drive the complex cellular and organismal process of mammalian ageing. The process appears to be hastened by environmental and behavioural factors including obesity, diabetes, end-stage renal disease and exposure to mutagens such as chemotherapy, ultraviolet light and tobacco smoke. Likewise, various genetic lesions that alter DNA metabolism and nuclear architecture have been associated with progeroid syndromes in humans such as ataxia telangiectasia, Werner syndrome and Hutchinson–Gilford syndrome[1]. Most compelling have been genetic studies in yeast, *Drosophila melanogaster*, *Caenorhabditis elegans* and mice, which have established crucial roles for the insulin–insulin-like growth factor-1 (IGF1)–AKT–forkhead transcription factor class O (FOXO) pathway (see the Review by Russell and Kahn in this issue) and oxygen-radical regulators (see the Opinion article by Pelicci and colleagues in this issue) as determinants of lifespan in these model systems. Although these disparate experimental observations suggest common molecular themes, human ageing in physiological terms remains an enigma.

In this Review, we suggest that some aspects of mammalian ageing result from an age-associated decline in the replicative function of certain tissue-specific regenerative cells — adult stem cells (FIG. 1). These rare and specialized cells are required for tissue replacement throughout the human lifespan, and appear to be characterized by a few specific physiological and biochemical properties (reviewed in REFS 2–4), particularly the capacity for self-renewal.

Recent evidence supports the model that stem cells in several tissues are largely retained in a quiescent state but can be coaxed back into the cell cycle in response to extracellular cues, even after prolonged periods of dormancy. Once stimulated to divide, stem cells yield undifferentiated progeny, which in turn produce differentiated effector cells through subsequent rounds of proliferation (BOX 1). This 'hierarchical' differentiation scheme makes sense from the perspective of organismal longevity — it permits the production of large numbers of differentiated cells from a single stem cell by combining subsequent steps in differentiation with proliferation[5,6]. Therefore, this approach balances the high rates of homeostatic proliferation that are required in tissues like the bone marrow and intestine with the long-term need to protect stem cells from mutagenic insult and carcinogenesis. Indeed, under homeostatic conditions, there is limited proliferative demand on the self-renewing stem cells themselves and so these cells divide infrequently, sparing stem cells the perils of DNA replication and mitosis. Additionally, as stem cells appear to be less metabolically active in their quiescent state, they may be subjected to lower levels of DNA-damage-inducing metabolic side products such as reactive oxygen species (ROS)[7].

Once believed to be restricted only to 'high-turnover' organs like the bone marrow and intestine, resident self-renewing cells are now thought to have a significant role in the homeostatic maintenance of many organs, including organs with lower turnover rates such as the brain and pancreatic islets[8]. Although there is disagreement as to the significance of such cells in these low-turnover

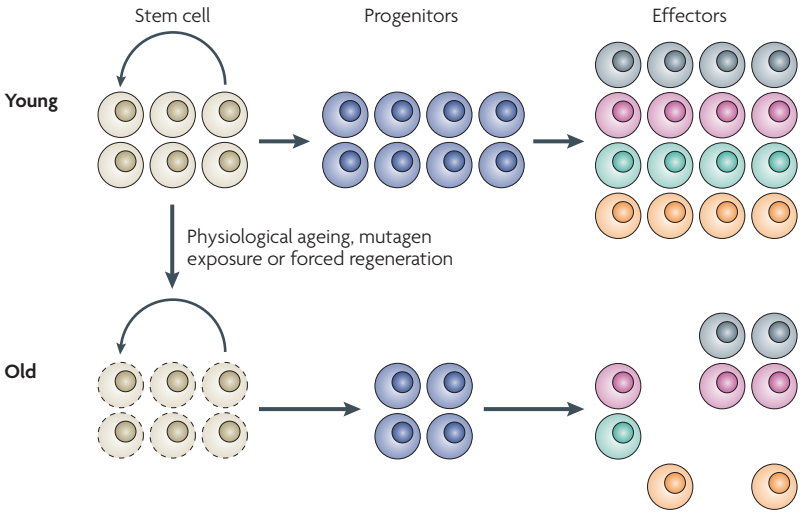


Figure 1 | **How stem cells age.** Stem-cell number and self-renewal (curved arrow) does not necessarily decline with ageing, but function — the ability to produce progenitors (blue) and differentiated effector cells (depicted in different colours) — does decline. Stem-cell ageing can result in some systems from the heritable accumulation of DNA damage, which can engage tumour-suppressor activation as the stem cell attempts to divide asymmetrically. DNA damage can occur stochastically with normal ageing as a result of exposure to external mutagens, or from increased proliferation as in forced regeneration (see main text).

organs in the adult human, their importance in rodents has been demonstrated experimentally. It is therefore reasonable to surmise that perhaps some characteristics of ageing — once thought to be degenerative — might reflect a decline in the regenerative capacity of resident stem cells across many different tissues.

Self-renewal comes with some danger for the organism; in particular, a risk of malignant transformation[4,9,10]. Unrepaired genetic lesions in stem cells are passed on to their self-renewing daughters and accumulate with ageing in this way. Functional mutations that provide a growth or survival advantage in turn produce positive selection for the mutant stem-cell clone, with full-fledged cancer resulting from the accumulation of multiple cancer-promoting events. To offset this possibility, stem cells appear to have evolved multiple reinforcing mechanisms that are aimed at maintaining genomic integrity beyond that of other proliferating cells (reviewed in REFS 2–4). When mutations occur despite these error-prevention capacities, potent tumour-suppressor mechanisms such as senescence and apoptosis exist to sense damaged stem-cell genomes with malignant potential and limit replicative expansion or cull such clones. This relationship between self-renewing cells and cancer raises the possibility that — while carrying out a beneficial, anti-cancer function — these tumour-suppressor mechanisms may inadvertently contribute to ageing by causing stem-cell arrest or attrition.

Here, we discuss recent refinements of this so-called 'cancer–ageing hypothesis', according to which cells within a tissue are compromised by these anti-cancer mechanisms (see also the Opinion article by Serrano and Blasco in this issue). Specifically, we reason that growth-inhibitory molecules such as the cyclin-dependent kinase inhibitor p16^{INK4a} and the tumour suppressor p53 exert their pro-ageing effects in part through their activation in specific self-renewing compartments such as tissue-specific stem cells. Further, we describe findings from a series of recently published human association analyses that suggest a link between the *INK4/ARF* locus (also called *CDKN2a* and *CDKN2b*) and the onset of distinct human age-associated phenotypes. These recent observations, together with the findings in genetic model systems, provide experimental support for the concept that the activation of tumour-suppressor mechanisms in self-renewing compartments contributes to the ageing processes in humans.

Do stem cells age?

A decline in replicative function with age is appreciable in many mammalian tissues. In most mammalian tissues, however, the inability to purify the resident stem cell to homogeneity, as well as the lack of adequate models to test the function of these cells, has made it difficult to determine if a decline in stem-cell function is indeed a cause of the degradation of the regenerative capacities that is seen in many organs with ageing.

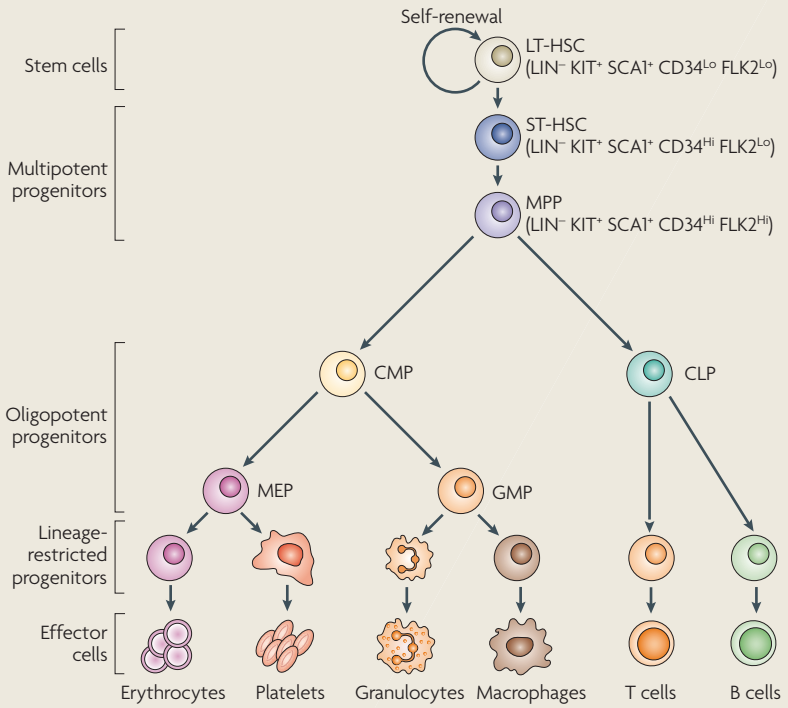
Ageing of haematopoietic stem cells. In the haematopoietic system, it is possible to purify haematopoietic stem cells (HSCs) to near-homogeneity and assay their function using validated assays. Therefore, the questions of whether and how stem cells age are presently best addressed in this system, with the caveat that stem-cell ageing may differ mechanistically in other tissues. Several effects of ageing on the blood organ are described in humans: decreased immunity[11], increased incidence of bone marrow failure and haematological neoplasia[12], and moderate anaemia[13,14]. Older individuals are more likely to suffer toxicity such as prolonged myelosuppression in response to traditional cytotoxic chemotherapy drugs, suggesting a reduced marrow regenerative capacity[15–17]. Particularly telling is the clinical observation that increased donor age in bone marrow transplantation is a predictor of transplant-related mortality[18–22], suggesting that the diminished reconstituting ability of HSCs from elderly donors is partly cell-autonomous. These observations suggest a clinically overt decay in HSC function with normal human ageing.

These correlative findings in humans are buttressed by related studies in rodents. A surprising finding has been that although HSC function clearly declines with age, the number of HSCs does not necessarily also decline. In some strains of mice, the HSC number actually expands with advancing age[7,23–27] and this age-dependent expansion of HSCs is a transplantable, cell-autonomous property of HSCs[7,25]. Moreover, as demonstrated by Harrison and colleagues, HSCs can be serially transplanted into sequential recipients and show persistent function for >8 years, thus exceeding the lifetime of the original donor animal[28]. These experiments have established that cell-autonomous, replicative HSC exhaustion does not necessarily occur during periods of normal ageing in some strains of inbred mice that are maintained under laboratory conditions.

Self-renewal
The capacity of replicating stem cells to generate daughter cells with the same biological and molecular profile that endows continued renewal potential. This can occur either asymmetrically when a stem cell produces another stem cell and a more differentiated daughter cell, or symmetrically when stem-cell division gives rise to two identical stem cells. Importantly, in mature organ systems, most cell-division activity that is responsible for tissue maintenance and expansion is not self-renewing.

Progeny
Along with progenitor cells, these are relatively undifferentiated cell types that are derived from asymmetric stem-cell division and lack the capacity to self-renew.

Box 1 | **The hierarchy of haematopoiesis**



Multipotent tissue-specific stem cells produce differentiated effector cells through a series of increasingly more committed progenitor intermediates. This tissue stem-cell-derived differentiation process has been best characterized in the haematopoietic system (see figure). Here, long-term haematopoietic stem cells (LT-HSCs) represent the 'true' stem cells that self-renew and produce multipotent progenitors (short-term (ST)-HSCs and, subsequently, multipotent progeny (MPP)) with no self-renewal capacity. These in turn give rise to oligopotent progenitors including the common lymphoid progenitor (CLP) and common myeloid progenitor (CMP), which then yields the granulocyte–macrophage progenitor (GMP) that differentiates into monocytes, macrophages and granulocytes, and the megakaryocyte–erythrocyte progenitor (MEP) that differentiates into megakaryocytes, platelets and erythrocytes. Similar hierarchies are also thought to exist in other stem-cell-containing tissues (for example, brain, gut, liver and lung).

A principal advantage of studies in the haematopoietic system is that stem and progenitor cells from this organ can be purified to near-homogeneity by surface markers. For example, LT-HSCs express low levels of lineage markers (LIN^-), high levels of the CD117/c-KIT receptor (KIT^+), high levels of a surface marker called SCA1 ($SCA1^+$), and low levels of another surface marker, CD34 ($CD34^{Lo}$). With each asymmetric division of a $LIN^-KIT^+SCA1^+CD34^{Lo}$ LT-HSC, there is the production of another LT-HSC and a multipotent daughter cell with limited renewal potential, ST-HSC, which has a similar surface immunophenotype to LT-HSC except that it has higher levels of CD34 ($CD34^{Hi}$). As ST-HSC in turn proliferate to form more differentiated MPP, they increase expression of another surface marker, FLK2. Development from oligopotent progenitors to mature blood cells proceeds through several intermediate progenitors that are not shown.

This is not to say, however, that the replicative function of HSCs is not limited by anti-cancer mechanisms with ageing. It is entirely possible that senescent HSCs change their surface immunophenotype (and therefore are no longer identified as HSCs by flow cytometry) or that the senescence and apoptosis mechanisms are not engaged until old HSCs attempt to divide asymmetrically. In the latter model, senescence or apoptosis could limit HSC function without decreasing the HSC number. Moreover, it is important to note that HSC numbers appear to decline with age in other inbred strains of mice[26,27,29–31], and that several external stimuli (for example, chemotherapy, ionizing radiation and so on) hasten stem-cell exhaustion in humans and mice[32–38]. Thus, genetically outbred mammals in the wild may or may not experience HSC exhaustion with ageing, and exposure to environmental stresses that are not normally encountered in the laboratory setting may further induce HSC exhaustion. Finally, independently of replicative function, HSCs exhibit cell-intrinsic, functional signs of ageing. Numerous studies have shown that ageing alters HSC function with regard to mobilization[39], homing[7,23,24,31,39] and lineage choice[7,24,31]. In particular, there is a loss of lymphoid lineage potential with a skewing toward myeloid lineages in HSCs from old mice, and old HSCs demonstrate reproducible changes in gene expression with age, including increased expression of myeloid lineage transcripts[7]. Therefore, the preponderance of evidence suggests that HSCs undergo cell-intrinsic ageing, although there is also emerging evidence that the ageing HSC microenvironment may influence HSC function in an extrinsic manner (see below).

Ageing in other self-renewing compartments. Several lines of evidence suggest that stem cells from other tissues suffer proliferative decline with advancing age (FIG. 1). For example, in rodents, a decline in the number of new neurons produced by neural stem cells (NSCs) with age or telomere dysfunction has been documented[40,41], as has the *in vivo* proliferation of NSCs[41–43]. This decline in the capacity for murine neurogenesis has been associated with a progressive Parkinsonian disease[41] and with an impairment of olfactory discrimination with ageing[44]. Likewise, hair greying has been linked to decreased melanocyte stem-cell maintenance, possibly in association with melanoblast senescence[45].

The capacity of the insulin-producing β-cell of the pancreatic islet to replicate in adult rodents throughout life has been known for decades, although the importance of this regenerative capacity with regard to the development of type 2 diabetes mellitus (also called adult-onset diabetes mellitus) has only recently been appreciated. The prevailing view had been that type 2 diabetes mellitus was a metabolic disease resulting largely from an age-associated decline in the ability of muscle and liver to respond to insulin (insulin resistance), but recent careful analyses of islet mass have challenged this view. Studies in the immediate post-mortem period have shown significant rates of β-cell production and apoptosis, even in aged adults, with an increased β-cell mass noted in obese individuals and a relatively reduced β-cell mass among adults with diabetes[46,47]. Therefore, the *de novo* synthesis of β-cells through self-renewal appears to be the predominant source of islet mass in adult humans. Islet replication appears to decline with human ageing[47], although β-cell replication has been reported in human patients up to 89 years of age[48]. Consistent with these findings in humans, a sharp decline in β-cell proliferation with ageing in mice has been recently described[49]. More than 1% of islet cells from young mice proliferate

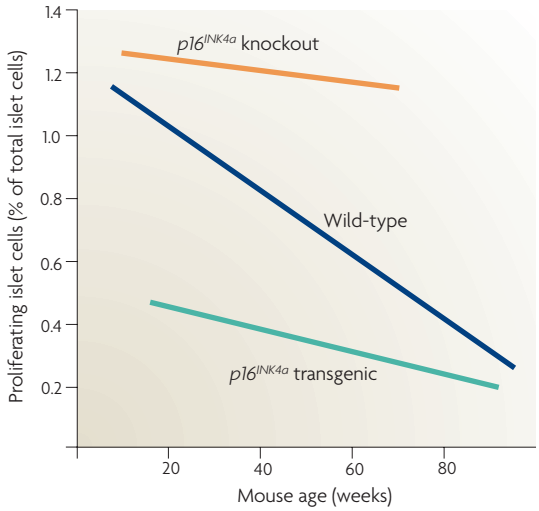


Figure 2 | **Proliferation of β-cells with age.** A sharp decline in pancreatic β-cell proliferation with ageing normally occurs in wild-type mice (blue). This decline is modulated by expression of the $p16^{INK4a}$ tumour suppressor: increased expression (in a transgenic line; green) correlates with reduced proliferation, whereas decreased activity (in the $p16^{INK4a}$ knockout line; orange) affords a resistance to β-cell ageing.

Senescence
A specialized form of growth arrest induced by various stressful stimuli including loss of telomere function, reactive oxygen species, some forms of DNA damage and activation of certain oncogenes or reactivation of tumour-suppressor genes. Senescence is characterized by several markers such as senescence-associated-β-galactosidase, alterations in chromatin structure (senescence-associated heterochromatic foci) and a marked increase in the secretion of several cytokines and other bioactive molecules (senescence-associated secretory phenotype).

Tissue-specific stem cell
A specialized cell found in many tissues of adults. These cells can replace themselves through self-renewal and are generally multipotent, in that they can give rise to progeny that can differentiate into multiple different cell types of the associated organ.

Multipotency
The ability to give rise to differentiated progeny of different specialized subtypes. However, some self-renewing cells (for example, pancreatic β-cells) have a narrow potential for differentiation, generating progeny similar to the parental cell. This type of self-renewing cell is termed a 'unipotent progenitor', which can be viewed as a special stem-cell subtype, at least in terms of long-term proliferative capacity. For convenience, in this Review the term 'stem cell' is applied to both types of adult self-renewing cells.

Telomere
A nucleoprotein complex at the end of chromosomes that maintains chromosomal integrity. It consists of many double-stranded 5′-TTAGGG-3′ repeats, a 3′-single-stranded overhang and associated telomere-binding proteins, which together generate a capped structure that is impervious to the actions of complexes that repair DNA damage.

under steady-state conditions, but this frequency declines by nearly tenfold after a year of ageing (FIG. 2). Therefore, rather than being merely a disease of insulin resistance, type 2 diabetes mellitus partly results from a relative failure of islet replication.

Cell-extrinsic ageing. These observations, however, do not make clear which islet-associated compartment is ageing: is it the β-cells themselves, a putative pancreatic stem cell or another tissue that influences β-cell proliferation in a cell non-autonomous manner? In fact, cell non-autonomous factors have been shown to have a major role in the regulation of stem-cell ageing in other systems. Rando and colleagues have shown that the ageing of a regenerative cell of muscle, the satellite cell, is influenced by the aged microenvironment[50]. Satellite cells from aged mice were 'rejuvenated' by exposure to a young blood supply, suggesting that the aged milieu, hormonal or otherwise, influences the replicative function of satellite cells. Likewise, while some aspects of the functional decline that characterizes ageing of HSCs has been shown to be cell-autonomous, the HSC niche is also expected to influence HSC ageing to some degree. For example, in mice with shortened telomeres, an age-dependent decrease was noted in the ability of recipient animals to support lymphopoiesis of transplanted HSCs from young mice with normal telomeres, and enhanced myeloid proliferation occurred when HSCs were transplanted into a microenvironment that harboured dysfunctional telomeres[51].

Although this distinction between cell-intrinsic and cell-extrinsic ageing is important, ageing is, ultimately, the summative property of the organism *in toto*. For example, the finding that serum from young mice enhances satellite-cell proliferation suggests that cell-intrinsic ageing of some other tissue occurs such that it elaborates (or fails to elaborate) the relevant serum factor that modulates the proliferative capacity of satellite cells. The ability to target genes conditionally in a tissue-specific and temporal manner will permit *in vivo* analyses to determine which compartments age intrinsically, versus those that age 'by proxy'.

What causes cells to age?

Although a few mechanisms have been suggested to explain cellular ageing, independent lines of evidence suggest that forms of DNA damage lead to the activation of tumour-suppressor mechanisms, such as senescence, to limit stem-cell function with increasing age.

DNA damage results in stem-cell attrition. The accumulation of damage to cellular macromolecules (for example, proteins and DNA) has been postulated to be a cause of cellular attrition with ageing[52]. DNA is subjected to spontaneous and extrinsic mutational events on a daily basis, and despite a formidable capacity for repair, some damaged DNA appears to evade repair and accumulates over time. Evidence in support of the notion that DNA damage attenuates stem-cell function with age has been provided by the study of HSCs from mice that harbour alterations in the DNA-damage response. Significant functional defects are seen in HSCs from mice that are deficient in DNA-repair proteins such as FANCD1(REF. 53), MSH2 (REF. 54) or ERCC1 (REF. 55). A mouse strain with a viable, hypomorphic allele of the DNA-repair protein DNA ligase IV that was recently identified through a mutagenesis screen exhibits a marked, age-induced decline in HSC number and function[56]. Additionally, Morales *et al.* have shown that mice with a mutant allele of RAD50, a member of the MRE11 DNA-repair complex, demonstrate profound bone marrow hypoplasia[57]. This effect can be rescued in the setting of ataxia-telangiectasia mutated (ATM) kinase deficiency, suggesting that the *Rad50* allele is hypermorphic and that excess DNA-damage signalling reduces HSC number and/or function. These data suggest that several forms of DNA repair are needed to maintain HSC genomic integrity, and that activation of a response to DNA damage compromises HSC function.

A demonstration of DNA damage in a tissue-specific stem-cell compartment with ageing comes from a recent report by Rossi and colleagues[58]. Using mice with germline deficiencies in DNA repair or telomere metabolism (Ku80-, XPD- and mTERC-knockout mice), the authors demonstrated a marked premature decline in the regenerative function per HSC in several assays. This study further noted the increased expression of markers of the DNA-damage response (for example, histone H2AX foci) in highly purified HSCs with normal physiological ageing even in wild-type mice. The authors noted an increase in apoptosis of progeny from old HSCs, but not a decline in the ability of old HSCs to proliferate in *in vitro* assays. These observations correspond well with a diminished capacity of the haematopoietic system

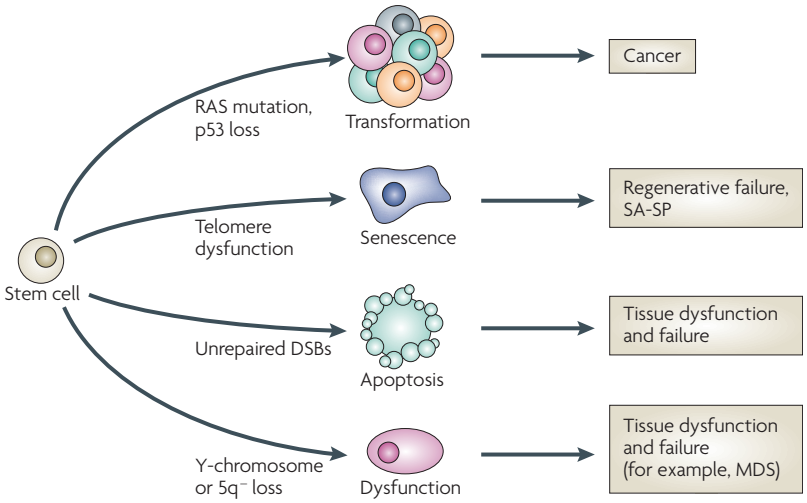


Figure 3 | **Fates of damaged stem cells.** A limited number of outcomes appear to be possible for stem cells with heritable DNA damage. Although it is likely that many mutational events do not lead to any alteration of stem-cell function, significant damage is expected to induce apoptosis, senescence, transformation or dysfunction. Importantly, these outcomes are not necessarily mutually exclusive; for instance, many genetic events associated with stem-cell dysfunction (for example, the oncogenic translocation that fuses *BCR* and *ABL* (forming the Tyr kinase BCR/ABL) in haematopoietic stem cells (HSCs)) are also associated with subsequent transformation. Examples of DNA lesions associated with each outcome are indicated. Mutations in RAS and the tumour-suppressor p53 are associated with transformation, whereas loss of the long arm of chromosome 5 ($5q^-$) or the Y-chromosome are associated with dysfunction of HSCs, manifesting as myelodysplasia (MDS). Unrepaired double-strand DNA breaks (DSBs) and telomere dysfunction induce apoptosis and senescence, respectively. SA-SP, senescence-associated secretory phenotype.

in telomere-dysfunctional mice to recover following chemotherapeutic challenge, as well as with a progressive neurodegenerative condition that is associated with decreased NSC reserves and function in the telomerase-knockout model[41,59]. Together, these results are consistent with the view that DNA damage accumulates with ageing in the HSC compartment, as evidenced by the accumulation of H2AX foci, and that this damage can be physiologically significant if unrepaired.

But what causes DNA damage in stem-cell compartments in adult mice? Recent data from Ruzankina *et al.*, who used a conditional allele for the DNA-damage response gene *Atr* (ataxia telangiectasia and Rad3-related), are worth considering in this regard[60]. Loss of *Atr* is toxic to proliferating cells[61] and when *Atr* was somatically excised in adult mice in a widespread manner by conditional inactivation, the vast majority of proliferating cells rapidly disappeared, producing marked intestinal atrophy and bone marrow hypoplasia 2 weeks after conditional activation. However, the animals survived this transient period of cell loss because rare stem cells that had not recombined the *Atr* allele replaced the lost cells. By 1 month after conditional activation, the mice appeared largely normal, with rapidly proliferating tissues that had been fully reconstituted by sporadic *Atr*-competent cells. Surprisingly though, these reconstituted mice then developed a marked progeroid phenotype a few months later, with osteopaenia, greying and loss of lymphoid and haematopoietic progenitors. Therefore, the excess regeneration that was forced to occur to reconstitute proliferative tissues after transient *Atr* inactivation in some proliferating cells produced a durable compromise of stem-cell function in *Atr*-competent cells (FIG. 1), although it is important to emphasize that the possible adverse effects of ATR deletion on the stem-cell niche may also have contributed to the accelerated ageing. This result is consistent with the view that such forced regeneration in response to homeostatic demands, even in the absence of external DNA-damaging agents, can be toxic to stem cells across many organ systems.

In the 'DNA-damage accrual' model of ageing, unrepaired (or improperly repaired) genomic damage accumulates with ageing in stem-cell compartments. At some point, accumulated damage perturbs normal stem-cell biology, driving stem cells to a few possible fates: transformation, senescence, apoptosis or dysfunction; for example, a loss of the ability to robustly produce progeny or an impaired potential for multilineage differentiation (FIG. 3). As this process proceeds with time, depleted and/or dysfunctional stem-cell compartments cannot match the regenerative needs of a given organ and homeostatic failure ensues. Likewise, if oncogenic DNA-damage-induced lesions accumulate, self-renewing clones that contain such lesions undergo positive selection, leading to cancer. Therefore, it is tempting to speculate that cancer and ageing are related endpoints of accumulating DNA damage within self-renewing compartments.

How telomere shortening could affect stem-cell ageing. There has been significant interest in the possibility that telomere dysfunction, a specialized form of DNA damage, contributes to the ageing of human stem cells. In the absence of adequate telomerase activity, telomere shortening inexorably occurs with proliferation, eventually triggering a change in telomere structure that is sensed by the cell as a DNA double-strand break. In humans, robust telomerase activity is predominantly restricted to germ-cell compartments, some somatic early stem-cell or progenitor compartments and proliferating lymphocytes.

Whereas telomere dysfunction in the setting of an intact DNA-damage response can serve as a potent tumour-suppressor mechanism, the role of telomere-mediated checkpoints in stem-cell ageing remains an area of active investigation. Mice have long telomeres relative to humans and, thus, telomere dysfunction appears not to be a major cause of stem-cell exhaustion in many strains of *Mus musculus*. Accordingly, in the telomerase-knockout mouse[62], the lack of telomerase activity *per se* produces a modest phenotypic effect in adult mice[59,63]. Murine telomere length can be made more limiting and reduced to a 'humanized' length by serial intercrossing of mice that are deficient in telomerase activity. In this experimental setting, telomere length becomes shorter with each successive generation until telomere dysfunction ensues with dramatic phenotypic effects, thus providing a model system to address the stem-cell issue.

Telomerase
A ribonucleoprotein complex that extends the ends of telomeres after replication by using telomerase reverse transcriptase (TERT) and an RNA template (TERC) that is part of the enzyme complex.

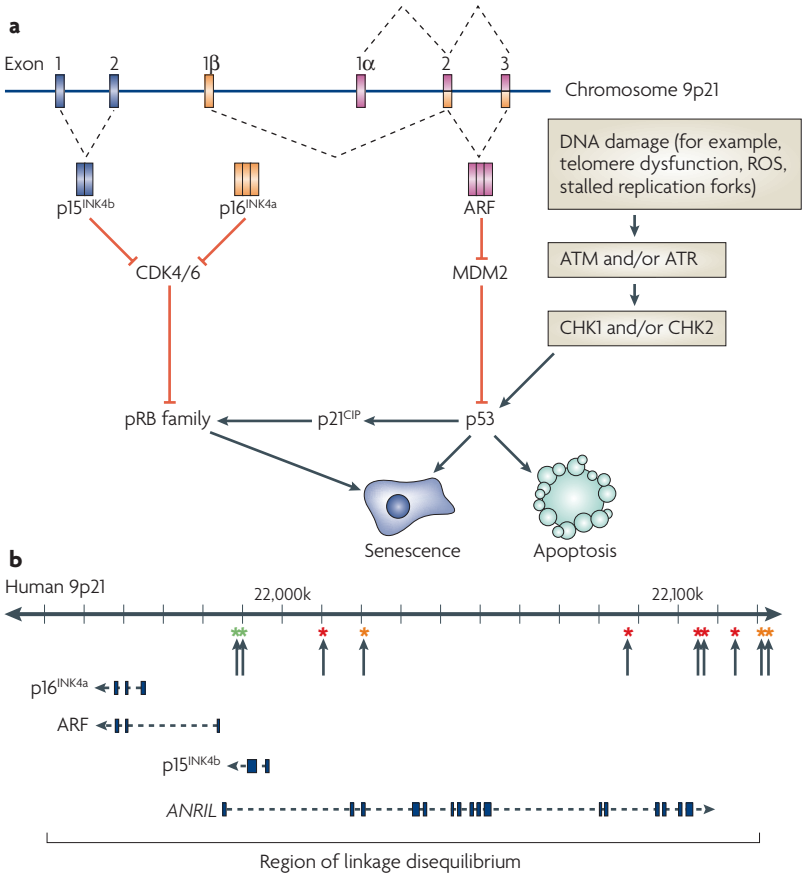


Figure 4 | **SNPs and age-related phenotypes at the *INK4a/ARF/INK4b* locus on human chromosome 9p21. a** | Proteins encoded by the *INK4/ARF* locus on chromosome 9p21 regulate the p53 and retinoblastoma protein (pRB) tumour-suppressor pathways to promote senescence (p53 and pRB-family activation) or apoptosis (p53 activation). Activation of p53 in response to several types of DNA damage also can occur via an ataxia-telangiectasia mutated (ATM)/ataxia telangiectasia and Rad3-related (ATR)-mediated pathway independent of ARF function. **b** | Single nucleotide polymorphisms that are significantly associated with the indicated phenotypes are shown (frailty, green; vascular heart disease, red; type 2 diabetes mellitus, orange). The open reading frames for $p16^{INK4a}$, ARF and $p15^{INK4b}$ are indicated, as is the *ANRIL* transcript. The large (>100 kb) region of linkage disequilibrium is also indicated. CDK4/6, cyclin-dependent kinases-4/-6; CHK1/2, checkpoint kinases-1/-2; MDM2, murine double minute-2, p53-binding protein; ROS, reactive oxygen species.

Animals with dysfunctional telomeres develop features of premature ageing caused by the activation of senescence and apoptosis mechanisms in certain self-renewing compartments such as HSCs, the intestinal crypt and the testes[59,63–66]. Deficiency of p53 rescues many of the stem-cell defects in mice that harbour defective telomeres, but does not extend the lifespan of these mice because of increased tumorigenesis[67,68]. By contrast, loss of $p21^{CIP}$, a p53 transcriptional target that potently inhibits the cell cycle (FIG. 4a), partially extends the longevity of mice with telomere dysfunction without increased tumorigenesis, and attenuates some of the proliferative defects that are seen in various stem-cell compartments of telomere-deficient mice[66]. Therefore, DNA damage induced by telomere shortening is partly sensed and managed through p53, and p53 exerts important anti-proliferative effects in stem cells through $p21^{CIP}$.

Senescence contributes to ageing. DNA damage and telomere dysfunction appear to activate the classical tumour-suppressor mechanisms of senescence and apoptosis. Senescence requires activation of the retinoblastoma (RB) and/or p53 proteins and expression of their regulators, most prominently $p16^{INK4a}$ and ARF (REFS 69–71) (FIG. 4a). The notion that senescence prevents cancer is well-supported and is not controversial (reviewed in REFS 72,73). The expression of markers of senescence such as senescence-associated β-galactosidase and $p16^{INK4a}$ markedly increases with ageing in many tissues from disparate mammalian species (reviewed in REF. 73; see also the Review by Campisi and D'Adda di Fagagna in this issue). Caloric restriction (CR) potently slows ageing in rodents, and CR and its related dietary changes retard or even abolish the age-induced increase in the expression of senescence markers, including the expression of $p16^{INK4a}$ (REFS 74–76). Provocatively, CR, similar to $p16^{INK4a}$ deficiency[77], enhances stem-cell function with ageing[78], which suggests the possibility that CR may slow ageing in mammals by decreasing the activation of senescence in self-renewing compartments.

A role for ROS in senescence bears particular relevance. ROS induces senescence in certain cell-culture systems[79,80] but may also have an important role in effecting the senescent phenotype[81]. In ATM-deficient mouse HSCs, increased ROS levels appear to compromise HSC function in part through a $p38^{MAPK}$-dependent activation of *Ink4a/Arf* expression[34,35], which can be attenuated by antioxidants. Likewise, HSCs that lack the FOXO transcription factors show diminished self-renewal and premature exhaustion under conditions of increased ROS production[82]. Therefore, FOXO transcription factors maintain HSC quiescence and survival, primarily via the regulation of physiological levels of ROS. While FOXO-deficient HSCs show ROS-dependent enhanced cell-cycle entry and increased apoptosis, increased ROS may also result in the accumulation of DNA damage and unscheduled activation of senescence mechanisms in this stem-cell compartment in the long term.

Although the expression of senescence markers is associated with ageing, this observation does not establish a causal relationship between senescence and ageing. Recent studies of self-renewal in HSCs, NSCs and pancreatic islet cells from $p16^{INK4a}$-deficient and $p16^{INK4a}$-overexpressing mice have begun to address this issue[43,49,77,83]. These results showed that increasing levels of $p16^{INK4a}$ are not only associated with ageing, but partly contribute to the age-induced replicative failure of these tissues. In all three compartments, $p16^{INK4a}$ deficiency attenuated the age-induced decline in proliferation and function. Likewise, overexpression of $p16^{INK4a}$ attenuated HSC function and islet proliferation in an age-dependent manner (FIG. 2). The effects of $p16^{INK4a}$ loss were consistent across these disparate self-renewing tissues, suggesting that $p16^{INK4a}$ can promote ageing in tissues that are developmentally distinct. However, the loss of $p16^{INK4a}$ did not completely abrogate the effects of ageing in any of the organs studied, indicating that $p16^{INK4a}$-independent ageing occurs in each of these compartments.

Related observations have likewise suggested a pro-ageing role for p53 and its effectors in mice[66,84,85] and humans[86]. The case for p53, however, appears to be more complicated because p53 and its downstream effectors such as $p21^{CIP}$ also have important roles in regulating the DNA-damage response. In fact, an intriguing recent study by Serrano and colleagues, who used carefully designed transgenic strains, has shown that increased ARF and p53 activity can increase the murine lifespan by preventing cancer without an attendant increase in organismal ageing[87]. Additionally, HSCs from $p21^{CIP}$-deficient mice demonstrate premature exhaustion[88], consistent with the notion that a p53- and $p21^{CIP}$-dependent cell-cycle pause in response to DNA damage may be important for stem-cell longevity *in vivo*. These results suggest that p53 activation can be both pro-ageing and anti-ageing depending on the nature and duration of the stress behind its activation.

Ageing and the *INK4/ARF* locus

The described links between stem-cell function and ageing predict that individuals who differentially regulate senescence-promoting mechanisms might exhibit different predispositions to cancer versus ageing. Accordingly, hypomorphic alleles that affect $p16^{INK4a}$ or p53 function and that are associated with increased types of cancer are well described (reviewed in REF. 73). Recent data also suggest that non-coding polymorphisms near the open reading frames (ORFs) of $p16^{INK4a}$, ARF and $p15^{INK4b}$ modify the onset of age-associated phenotypes in humans. In a remarkable series of recent studies, single nucleotide polymorphisms (SNPs) that are very close (<120 kb) to the *INK4/ARF* locus were associated with frailty[89], type 2 diabetes mellitus[90–92] and vascular heart disease[93–95] (FIG. 4b). The identified SNPs may not be the actual variants that convey disease susceptibility or resistance, and several classes of lesions (for example, small insertions and deletions) would not have been detected by the chosen approach. Different SNPs near the locus have been associated with these phenotypes, and at least a few of the associated SNPs are not in linkage disequilibrium with each other, which suggests that more than one polymorphism near the locus influences these ageing phenotypes.

Specifically, three research consortia that undertook genome-wide association studies across large, carefully annotated patient samples have reported an association between type 2 diabetes mellitus and several SNPs near *$p16^{INK4a}$* (REFS 90–92). The *INK4/ARF*-associated SNPs were independently identified and have common risk allele frequencies and considerable effect sizes. This result suggests that expression of $p16^{INK4a}$, ARF and/or $p15^{INK4b}$ may be a significant determinant of susceptibility for type 2 diabetes mellitus. This model fits with the newly appreciated importance of islet replication in human type 2 diabetes mellitus and the effect of $p16^{INK4a}$ on islet regeneration *in vivo*[49]. Also using unbiased, genome-wide approaches, three consortia recently described SNPs near the *INK4/ARF* locus that correlate with myocardial infarction and atherosclerotic heart disease (ASHD) in large human cohorts[93–95]. Importantly, these SNPs did not correlate with lipids, glycaemia or body mass index, implying that they influence ASHD independently of these known metabolic characteristics. That is, the ASHD SNPs do not appear merely to be type 2 diabetes mellitus SNPs despite the strong link between diabetes and vascular disease. A mechanistic basis for this association has been supplied by several reports that suggest links between senescence and age-related diseases of the cardiovascular system[96–98]. Given these results, it will be interesting to determine the effects of $p15^{INK4b}$, $p16^{INK4a}$ and ARF on atherosclerosis in murine models.

Regulatory polymorphisms that reside >1 Mb from the related ORFs are well established[99] and, therefore, the *INK4/ARF* locus appears well within the range of such regulatory elements. Although there has been a recent description of an apparently non-protein-coding transcript in this region (*ANRIL*[100]; FIG. 4b), there does not appear to be another protein-coding transcript near these SNPs. Therefore, in light of the murine genetic studies that link *Ink4a/Arf* and stem-cell function, proteins encoded by the locus are the strongest candidates to mediate the effects of these polymorphisms on the incidence of these common human diseases that are associated with ageing.

Outstanding questions

We believe that the data derived from disparate rodent and human experimental systems support the view that a decline in the regenerative function of stem cells with age contributes to mammalian ageing and age-associated disease. Given this model, a few crucial questions facing the field remain.

Does telomere dysfunction affect human ageing? An important issue is whether sufficient telomere attrition occurs in human self-renewing compartments during physiological ageing to activate a DNA-damage response and the subsequent compromise of replicative function. We feel that the current data do not yet provide a clear answer. Humans who harbour short telomeres because of congenital deficiencies of components of the telomerase complex (the RNA component TERC, telomerase reverse transcriptase (TERT) or dyskerin) develop an age-related failure of bone marrow or lung[101–105]. Moreover, telomere shortening in the liver precedes the onset of liver failure (cirrhosis) in patients with chronic hepatitis[106–109], an association that has been validated in the telomerase-deficient mouse[110]. A few studies have demonstrated a correlation between telomere length in peripheral blood lymphocytes (PBLs) and the onset of certain diseases that are associated with ageing. Such studies in non-neoplastic diseases have shown that PBL telomere lengths can provide a biomarker that forecasts the development of atherosclerosis[111,112], cancer risk[113,114] and mortality[115]. These results support a connection between telomere dysfunction and human ageing.

By contrast, arguments can be made against telomere-based ageing in humans. Murine ageing appears to have many similarities to human ageing, yet we believe that it occurs largely independently of telomere length.

Single nucleotide polymorphism
A common, single-base difference in a gene among individuals within a species.

Frailty
A clinically validated, functional measure used in clinical geriatrics. It is scored as a continuous variable using a series of routine, easily measured tests such as gait speed. Frail individuals are less able to live independently, are more likely to harbour co-morbid illnesses, and exhibit increased mortality.

Linkage disequilibrium
(LD). A measure of genetic associations between alleles at different loci, which indicates whether allelic or marker associations on the same chromosome are more common than expected. Loci are generally considered to be in strong LD if their correlation is higher than a pre-defined cut-off (for example, 0.8).

Additionally, although telomere length shortens with human ageing in many tissues, overall telomere length does not linearly correlate with age, with the most rapid shortening occurring by young adulthood[102,105,116]. It is possible, however, that mean telomere length is a poor indicator of telomere status because the shortest telomere appears to be capable of activating the DNA-damage response[117].

Although the observations of bone marrow failure and pulmonary fibrosis in patients with defective telomerase components establishes that telomere dysfunction can cause human disease, some individuals from such kindreds who harbour the defective allele do not demonstrate any overt phenotype. In fact, heterozygous TERT and TERC germline mutations in humans show strong 'anticipation'[102,104,105], which means that the phenotypic effects of the mutation are more severe in subsequent generations; this is analogous to findings in telomerase-deficient mice. Therefore, TERT or TERC deficiency is most phenotypically pronounced in patients that also inherit fore-shortened telomeres from their parents, suggesting that telomerase deficiency *per se* may not be sufficient to induce overt age-associated pathology. Importantly, however, studies of the unaffected carriers of these kindreds have so far not been sufficient to exclude subtle, age-associated phenotypes, and phenotypic consequences of telomerase deficiency may be noted in such individuals with more careful observation. Considered in aggregate, we believe that the data are consistent with both telomere-independent and telomere-dependent ageing of human stem cells.

How do senescence factors contribute to ageing? The simplest explanation for how $p16^{INK4a}$ limits replicative function with age would be that the induction of $p16^{INK4a}$, in response to senescence-promoting cues, intrinsically limits replication by inducing senescence or at least decreasing cell-cycle entry. For example, pancreatic β-cell replication is known to require cyclin-dependent kinase-4 (CDK4) activity[118,119], the biochemical target of $p16^{INK4}$. Because $p16^{INK4a}$ accumulates with physiological ageing in human and rodent islets[49,120], and the loss of $p16^{INK4a}$ augments islet proliferation in an age-dependent way[49], $p16^{INK4a}$ might directly limit the replication of self-renewing cells with ageing. This cell-autonomous model is further supported by the fact that the enhanced function of $p16^{INK4a}$-deficient HSCs is seen even when such cells are transplanted into $p16^{INK4a}$-competent recipient mice[77,83]. Importantly, however, $p16^{INK4a}$ expression increases significantly with ageing in lineage-negative bone marrow cells (of which <0.1% are true stem cells), but only modestly in highly purified long-term (LT)-HSCs (BOX 1) from old mice; LT-HSCs from old mice maintain the ability to replicate *in vitro*, which suggests that they are not senescent[24,58]. Therefore, although genetic approaches using $p16^{INK4a}$-deficient and $p21^{CIP}$-deficient mice have suggested an important effect of these proteins in regulating HSC biology with age, experiments that rely on the analysis of highly purified LT-HSC are not consistent with the accumulation of senescent LT-HSCs with age.

To reconcile these observations, we favour the model that the activation of $p16^{INK4a}$ and other senescence-promoting pathways (for example, ARF–p53, FOXO–ROS and so on) are repressed in true stem-cell compartments, but that such pathways may limit the function of stem cells as they asymmetrically produce progeny. In accordance with this hypothesis, diverse stem-cell compartments require the persistent ability of Polycomb group proteins such as BMI1 to repress the senescence-promoting activities of the *Ink4a*/*Arf* locus[121–125] (FIG. 4a). Absence of BMI1 does not lead to an increase in the numbers of hypofunctional LT-HSCs but instead causes a marked depletion of LT-HSCs, which is partly rescued by concomitant *Ink4a*/*Arf* inactivation[126]. Therefore, *Ink4a*/*Arf* activation in *Bmi1*-null mice does not produce 'senescent' LT-HSCs, at least on the basis of surface immunophenotype, but instead decreases their frequency. Given this observation, it is likewise not surprising that senescent LT-HSCs do not accumulate with age, even though $p16^{INK4a}$ activation has a causal role in the decline of HSC function with age[77]. Accordingly, the increase in $p16^{INK4a}$ expression in lineage-negative bone marrow cells from old mice probably reflects either the accumulation of senescent progeny or senescent cells that were formerly LT-HSCs but have changed their surface immunophenotype.

Why are 9p21 SNPs linked to age-associated diseases? The recent findings from human association studies correlating loci that are near the *INK4a*/*ARF* locus on chromosome 9 to the development of type 2 diabetes mellitus, ASHD and frailty are striking. Some might argue that type 2 diabetes mellitus and ASHD are ageing-associated diseases rather than ageing *per se*. Although this is an important distinction, we feel that any model of human ageing that does not account for these causes of significant human morbidity is less useful and, therefore, we believe their potential association with $p16^{INK4a}$, $p15^{INK4b}$ and/or ARF expression is of relevance in this discussion.

It should be noted that, despite the data that support links between $p16^{INK4a}$ and age-associated declines in stem-cell function, it remains possible that these SNPs do not correlate with altered regulation of *INK4*/*ARF* products. It could be that there is an unappreciated protein-coding ORF near the locus, that a non-protein coding transcript such as *ANRIL* (FIG. 4b) encodes regulatory RNAs, or that a polymorphism in this region exerts long-range (>1 Mb) regulation of another protein-coding or microRNA transcript. Studies in humans and mice to understand whether this correlation reflects an effect of *INK4*/*ARF*-based tumour-suppressor mechanisms on human ageing are moving at a rapid pace. Such efforts are hampered, however, by a large region of linkage disequilibrium that comprises the *INK4a*/*ARF* locus as well as almost all of the disease-correlated SNPs (FIG. 4b), which implies that the relevant genetic regulatory events could be 'hiding' anywhere within a large region around $p15^{INK4b}$. Regardless of the mechanistic basis, however, as the minor allele frequencies of the relevant SNPs in each of these reports are large (>10%), these polymorphisms appear to have a major role in determining the onset of these highly common, age-induced phenotypes.

Conclusions

In summary, we believe the data suggest that we grow old partly because our stem cells grow old as a result of mechanisms that suppress the development of cancer over a lifetime. In this regard, our self-renewing stem cells appear to grow old because of heritable intrinsic events, such as DNA damage, but also due to cell-extrinsic events such as alterations in their supporting niches. Anti-cancer mechanisms such as senescence and apoptosis, which rely on telomere shortening and/or p53 and $p16^{INK4a}$ activation, appear to promote ageing just as their failure is associated with cancer. We believe that a further, more precise mechanistic understanding of this process will be required before this knowledge can be translated into human anti-ageing therapies. For the time being, the most prudent, clinically validated advice appears still to be: don't smoke, eat reasonably and take exercise.

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Competing interests statement
The authors declare no competing financial interests.

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Atr
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
ataxia telangiectasia | Hutchinson–Gilford syndrome | type 2 diabetes mellitus | Werner syndrome
UniProtKB: http://ca.expasy.org/sprot
ATM | $p15^{INK4b}$ | $p16^{INK4a}$ | p53 | RB

FURTHER INFORMATION
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