REVIEW ARTICLE

The Stem Cell Hypothesis of Aging

Anna Meiliana[1,2] and Andi Wijaya[1,2*]

[1]Post Graduate Program in Clinical Biochemistry, Hasanuddin University, Makassar
[2]Prodia Clinical Laboratory, Jakarta
*Address correspondence to this author at: Prodia Clinical Laboratory, Jl. Cisangkuy No 2, Bandung
E-mail: anna_m@prodia.co.id, andi_w@prodia.co.id

Abstract

BACKGROUND: There is probably no single way to age. Indeed, so far there is no single accepted explanation or mechanisms of aging (although more than 300 theories have been proposed). There is an overall decline in tissue regenerative potential with age, and the question arises as to whether this is due to the intrinsic aging of stem cells or rather to the impairment of stem cell function in the aged tissue environment.

CONTENT: 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 cells types with advancing age. This decrease regenerative capacity appears to pointing to the "stem cell hypothesis of aging".

SUMMARY: Recent evidence suggested that we grow old partly because of our stem cells grow old as a result of mechanisms that suppress the development of cancer over a lifetime. We believe that a further, more precise mechanistic understanding of this process will be required before this knowledge can be translated into human anti – aging therapies.

KEYWORDS: Stem cells, senescence, telomere, DNA damage, epigenetic, aging.

Introduction

Nascimur uno modo, multis morimu ("in one way we are born, in many ways we die"), and there is probably no single way to age. Indeed, so far there is no single accepted explanation or mechanism of aging (although more than 300 theories have been proposed (1), and controversy reigns on whether aging is the expression of a specific genetic programme or the simple consequence of a lifelong accumulation of random molecular damage (2).

A diminished capacity to maintain tissue homeostasis is a central physiological characteristic of aging. As stem cells regulate tissue homeostasis, depletion of stem cell reserves and/or diminished stem cell function have been postulated to contribute to aging (3). It has further been suggested that accumulated DNA damage could be a principal mechanism underlying age-dependent stem cell decline (4).

Stem cell functional capacity was severely affected under conditions of stress, leading to loss of reconstitution and proliferative potential, diminished self-renewal, increased apoptosis and, ultimately, functional exhaustion. Moreover, evidence that endogenous DNA damage accumulates with age in wild-type stem cells. These data are consistent with DNA damage accrual being a physiological mechanism of stem cell aging that may contribute to the diminished capacity of aged tissues to return to homeostasis after exposure to acute stress or injury (5).

Many theories have been put forth to explain the decline of cell and tissue function with age, but a main challenge for researchers who study aging is to distinguish among potential causal influences, virtually all of which

interact with one another and lead to organismal aging. The free-radical theory of aging proposes that reactive oxygen species, which are by-products of normal metabolism, are responsible for damage to many cellular components, including DNA (6).

Several mechanisms of DNA repair that are essential for healthy tissues and long life (7) have evolved in cells of higher organisms. In humans or mice, mutations in genes encoding DNA repair enzymes may lead to dramatic increases in the incidence of cancer and the shortening of lifespan (8).

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 aging (9).

What is Aging

Aging is commonly characterized as a progressive, generalized impairment of function, resulting in an increasing vulnerability to environmental challenge and a growing risk of disease and death (10). Aging occurs at least in part as a consequence of this imperfect maintenance, rather than as a genetically programmed process. Although aging may involve damage to various cellular constituents, the imperfect maintenance of nuclear DNA likely represents a critical contributor to aging. Unless precisely repaired, nuclear DNA damage can lead to mutation and/or other deleterious cellular and organismal consequences. Damage to both nuclear DNA, which encodes the vast majority of cellular RNA and proteins, and mitochondrial DNA have been proposed to contribute to aging (11).

Ecological factors such as hazard rates and food availability influence the trade-offs between investing in growth, reproduction, and somatic survival, explaining why species evolved different life spans and why aging rate can sometimes be altered, for example, by dietary restriction (10). Almost every aspects of organism's phenotype undergoes modification with aging, and this phenomenological complexity has led, over the years, to a bewildering proliferation of ideas about specific cellular and molecular causes. An attempt by Medvedev to rationalize the multiplicity of hypotheses resulted in a listing more than 300 "theories" of aging. Fortunately, recent advances have resulted in significant simplification of the theoretical underpinnings of aging research (12)

There are many sources of DNA damage. In addition to external sources, such as ionizing radiation and genotoxic drugs, there are also cell – intrinsic sources, such as replication errors, spontaneous chemical changes to the DNA, programmed double – strand breaks (DSBs) (in lymphocyte development), and DNA damaging agents that are normally present in cells. The latter category includes reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, hydrogen peroxide, nitric oxide, and others. Major sources of cellular ROS production are the mitochondria, peroxisomes, cytochrome p450 enzymes, and the antimicrobial oxidative burst of phagocytic cells. ROS can cause lipid peroxidation, protein damage, and several types of DNA lesions: single – and double – strand breaks, adduct, and crosslinks. The situation in which ROS exceed cellular antioxidant defenses is termed oxidative stress. As normal byproducts of metabolism, ROS are a potential source of chronic, persistent DNA damage in all cells and may contribute to aging (13).

ROS and many other DNA-damaging agents can cause cells to enter a state of irreversible cell cycle arrest accompanied by characteristic morphologic and functional alterations, termed senescence (14). The induction of senescence depends on pathways involving the p53 and Rb proteins. Cellular senescence has been best characterized in cultures of human fibroblasts and mouse embryo fibroblasts (MEFs), which cease expanding after repeated passage in culture, a process termed replicative senescence. Replicative senescence has been employed as a cellular model for aging; many mutations in DNA repair genes that cause premature aging phenotypes also confer premature replicative senescence (7).

At the cellular level, it has been known for 40 years that normal, differentiated cells such as fibroblasts have a limited division potential before undergoing so-called "replicative senescence." This is in contrast to malignantly transformed cells, which can divide indefinitely. In human cells, the difference is largely due to the presence or absence of telomerase, suggesting that normal cells may be programmed to undergo senescence, perhaps as a protection against tumor formation (10). Stress-induced DNA damage appears to be more important than the end replication problem for determining the rate of telomere erosion (15).

Two general models have been proposed to explain how cellular senescence may contribute to aging (16). First, senescence of progenitor or stem cells themselves could impair tissue renewal. In this regard, the *Polycomb* group repressor Bmi1 appears to control levels of hematopoietic stem cells via negatively regulating the induction of senescence specifically in these stem cells. Second, senescent cells secrete proteases and other factors

that may disrupt tissue function. In this regard, senescence has a complex relationship with neoplasia. Senescence has been postulated to occur as a tumor suppressor mechanism, whereby cells that have undergone a genotoxic insult and therefore possess the potential for neoplastic transformation enter a state in which they are incapable of dividing. However, senescent stromal cells can actually promote the growth of epithelial cancers, malignancies that occur with increased incidence in the elderly. Senescence has been offered as an example of "antagonistic pleiotropy," a process that is beneficial in young organisms but deleterious later in life: senescence suppresses cancer by preventing potentially tumorigenic cells from dividing but may potentially contribute to organ dysfunction in the aged through a variety of mechanisms, perhaps even contributing to neoplasia in this setting. However, a causal relationship between cellular senescence and organismal aging has yet to be proved (16).

The hypothesis that nuclear DNA, a critically important cellular constituent that cannot be replaced, is an important target of age-related change is supported by evidence that nuclear DNA damage and mutations accumulate with age. While ROS are likely to be one important source of this damage, there are numerous other cellular and environmental sources of damage, and the impact of such lesions may be enhanced by age-related compromise of DNA repair. In the latter context, most premature aging syndromes are caused by mutations in genes encoding proteins involved in DNA repair. Accumulation of mutations in critical genes may be one general mechanism by which compromised DNA repair could contribute to aging. In addition, p53-mediated senescence and apoptosis, in response to DNA damage, also likely contribute to aging (11).

In the three main pathways of conserved pro – aging pathways, the Insulin/insulin – like growth factor 1 signaling (IIS). Target of Rapamycin (TOR) and mitochondrial pathway, are indicated. The pro-aging activities of these pathways are conserved across species, with energy sensors, such as AMPK, as potentially important hubs in the complex networks that integrate them. However, it is important to note potential dissimilarities among species as well. Most, if not all, defects in the mitochondrial respiratory chain are lethal or cause disease in humans[62], but can increase lifespan in nematodes or

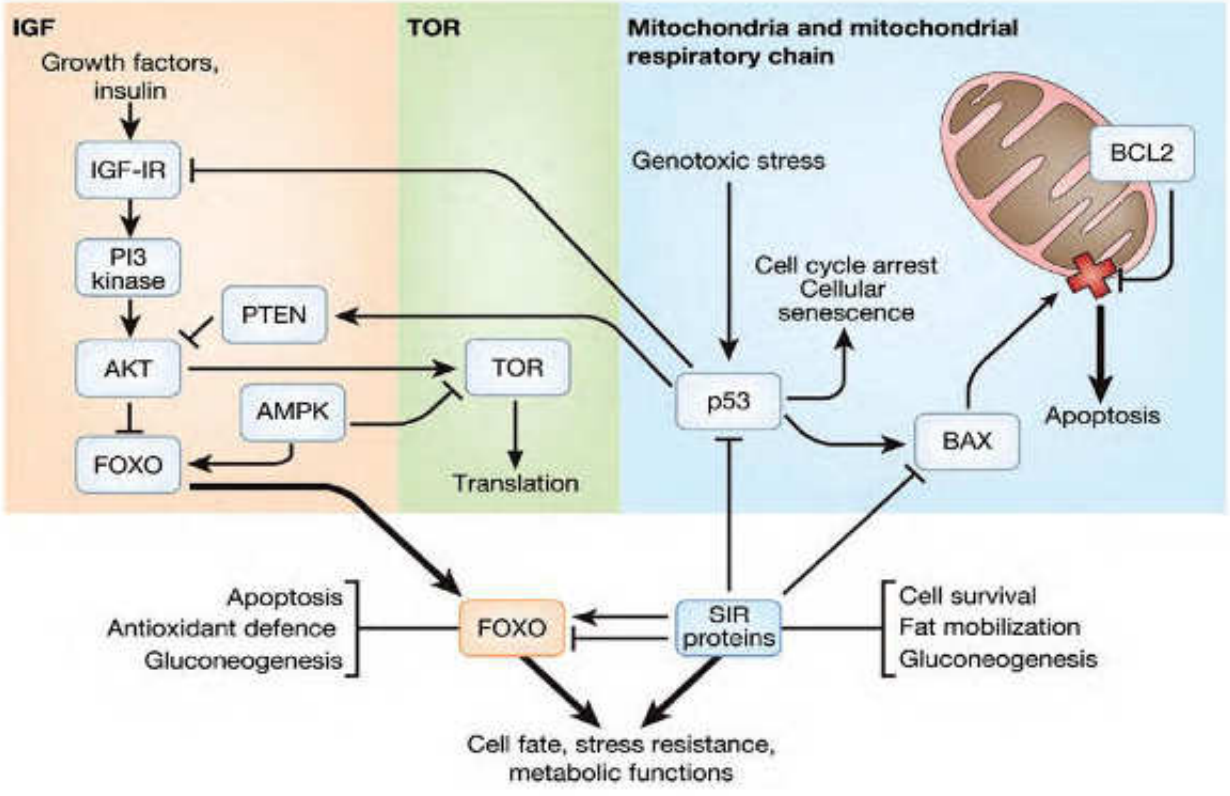

Figure 1. Potentially conserved pro – aging pathways (Adapted with permission from Nature Publishing Group).

yeast. In mammals, mitochondria play an important part in signalling apoptosis, which can either drive or retard aging, depending on the cell type. There is evidence that many longevity signals converge on members of the FOXO and sirtuin protein families, which can interact. Note that SIR proteins can both activate and repress FOXO. Moreover, the effects of FOXO and SIR2 in cells can be either beneficial (for example, increasing antioxidant defence) or detrimental (for example, apoptosis), and may or may not promote organismal survival. For example, in mammals, SIRT1 dampens apoptosis by repressing FOXO, but also by repressing BAX activation, thereby preventing its oligomerization into the mitochondria outer membrane (cross), which normally triggers permeabilization of the membrane and release of soluble apoptogenic factors, such as cytochrome c, into the cytosol. Apoptosis can be beneficial, for example, by eliminating damaged cells and preventing cancer, or can be detrimental, by eliminating irreplaceable cells, such as neurons (17).

In theory, interventions could be designed to alter the orchestrated networks of cell–cell interaction to increase lifespan. This is essentially what evolution has done to produce long-lived species. The question is, can we mimic the evolutionary process to the extent that senescence becomes essentially negligible? At this stage, the answer must be that we do not know. Although there is no scientific reason for not striving to cure aging—similar to what we profess to do for cancer and other diseases—our current understanding makes it impossible to assert that indefinite postponement is feasible. Rather, we need to use the current momentum to intensify research aimed at resolving major outstanding questions that hinder a more complete understanding of basic aging mechanisms and their relationship to disease. Only this will allow us to generate sophisticated, integrated strategies to increase human health and lifespan (17).

Theories of Aging

Nearly a century ago it was noted that animals with higher metabolic rates often have shorter life spans. These observations led to the formulation of 'the rate-of-living hypothesis', which states that the metabolic rate of a species ultimately determines its life expectancy. Initially, the mechanistic link between metabolism and aging was unknown.

At the molecular level, evidence suggests that several of the most important mechanisms involve damage to macromolecules. Some of the major theories that have been proposed to explain aging are the following:

SOMATIC MUTATION THEORY

Numerous studies have reported age-related increases in somatic mutation and other forms of DNA damage, suggesting that the capacity for DNA repair is an important determinant of the rate of aging at the cell and molecular level. There is a general relationship between longevity and DNA repair. This is particularly well illustrated by studies on the enzyme poly(ADP-ribose) polymerase-1 (PARP-1), which is a key player in the immediate cellular response to stress-induced DNA damage (20,21).

TELOMERE LOSS THEORY

In many human somatic tissues, a decline in cellular division capacity with age appears to be linked to the fact that the telomeres, which protect the ends of chromosomes, get progressively shorter as cells divide. This is due to the absence of the enzyme telomerase, which is normally expressed only in germ cells (in testis and ovary) and in certain adult stem cells (23).

MITOCHONDRIAL THEORY

An important connection between molecular stress and aging is suggested by the accumulation of mitochondrial DNA (mtDNA) mutations with age (23). Age-related increases in the frequency of cytochrome c oxidase (COX) deficient cells, which are associated with mtDNA mutation, have been reported in human muscle (24,25), brain (26,27), and gut (27). Cells in which mtDNA mutation reaches a high level are likely to suffer from impaired ATP production, resulting in a decline in tissue bioenergenesis.

ALTERED PROTEINS THEORY AND WASTE ACCUMULATION THEORY

Protein turnover is essential to preserve cell function by removing proteins that are damaged or redundant. Age-related impairment of protein turnover is indicated by the accumulation over time of damaged proteins, and there is evidence that an accumulation of altered proteins contributes to a range of age-related disorders, including cataract, Alzheimer's disease, and Parkinson's disease. With aging, there is evidence for functional declines in the activities of both proteasomes (28) and chaperones (29). These declines may be part of a more general failure, through overload, of cellular "waste disposal" processes (30).

NETWORK THEORIES OF AGING

Much of the early proliferation of aging theories arose from

a tendency to see the different hypotheses as competing to explain how aging occurs. The disposable soma theory suggests that multiple kinds of damage will accumulate in parallel within cells, since the same logic limits the investment in each of a wide range of maintenance and repair functions. Although the multiplicity of aging mechanisms is now widely acknowledged, the reductionist nature of experimental techniques means that, in practice, most research is still narrowly focused on single mechanisms.

This has led to recent initiatives to develop "'network" theories of aging in which the contributions of the various mechanisms are considered together, thereby allowing for interaction and synergism between different processes (31). Furthermore, such network models can highlight important differences between "upstream" mechanisms that set a process in train and "end stage" mechanisms that dominate the cellular phenotype at the end of its life (32).

In summary, current theoretical understanding suggests that, as cells age, they tend to accumulate damage. The rate at which damage arises is dictated, on the average, by genetically determined energy investments in cellular maintenance and repair, at levels optimized to take account of evolutionary trade-offs. Long-lived organisms make greater investments in cellular maintenance and repair than short-lived organisms, resulting in slower accumulation of damage. In order to manage the risk presented by damaged cells, particularly the risk of malignancy, organisms have additionally evolved mechanisms, such as tumor suppressor functions, to deal with damaged cells. The actions of such "coping" mechanisms will frequently involve a second tier of trade offs (10).

Aging is, above all, about the failure of living systems to keep going. Such systems do not, as a rule, give up the struggle easily, and we may anticipate that, in learning how aging erodes and eventually overwhelms our survival mechanisms, we will learn a great deal more about how these mechanisms are organized (10).

H_2O_2 as Determinant of Lifespan

The free-radical or oxidative stress hypothesis is one of the most accepted theories of aging. It states that oxidizing species are produced during aerobic metabolism, which consequently causes molecular damage and, over time, cell and tissue dysfunction, ultimately increasing the risk of disease.

ROS induce cell senescence and apoptosis; and finally, ROS, senescence and apoptosis are mechanistically linked to aging-associated degenerative diseases (2). Despite its popularity, one fundamental aspect of this theory still remains puzzling: how and why are dangerous pro-oxidant species generated during oxidative metabolism? It is generally thought that ROS are generated accidentally as by-products of the aerobic metabolism, and mitochondria are the major source of intracellular ROS. Recent findings, however, demonstrate that one specific ROS, hydrogen peroxide (H_2O_2), is produced by mitochondria through a specialized enzyme to control cellular growth and death. As genetic models of longevity indicate that H_2O_2 is a crucial determinant of lifespan, aging should be considered as the expression of a selected genetic programme that generates H_2O_2 as a signalling molecule (33).

Recently, it became evident that H_2O_2 is directly implicated in the physiological regulation of the signal transduction events that are triggered by activated growth-factor receptors. The intracellular level of H_2O_2 is regulated by various growth factors (such as epidermal growth factor (EGF), platelet derived growth factor (PDGF) and insulin), and H_2O_2 itself inhibits crucial phosphatases that are involved in the attenuation of signal propagation from activated growth-factor receptors. It appears, therefore, that H_2O_2 directly regulates the intensity of growth-factor signaling in cells (34).

H_2O_2 can function as a signaling molecule owing to its ability to induce fully reversible protein modifications. It has been demonstrated that H_2O_2 directly oxidizes cysteinyl thiol, inducing the formation of disulphide bonds and sulphenic acids, and induces glutathionylation of Cys residues or sulphoxidation of Met residues in various targets.

H_2O_2 functions as a signaling molecule in the intracellular propagation of both physiological and oncogenic growth signals. The induction of cell proliferation by several growth factors (such as EGF, PDGF, nerve growth factor (NGF) and insulin) correlates with a transient increase of intracellular H_2O_2, whereas antioxidant treatments prevent DNA synthesis (35). Similarly, cellular transformation following the expression of activated oncogenes (such as mutated Ras (36) or overexpressed *myc*) (37) is associated with increased intracellular H_2O_2 and is prevented by treatment with antioxidants (38,39). Furthermore, H_2O_2 mediates angiogenic signalling and has been implicated in the so-called angiogenic switch, which allows non-invasive and poorly vascularized tumours to become highly invasive and angiogenic tumours (through direct activation of the transcription factor hypoxia-inducible factor (HIF) (40).The function of H_2O_2 as a mitogenic or angiogenic signaling molecule is supported by its documented activity on various well established signal

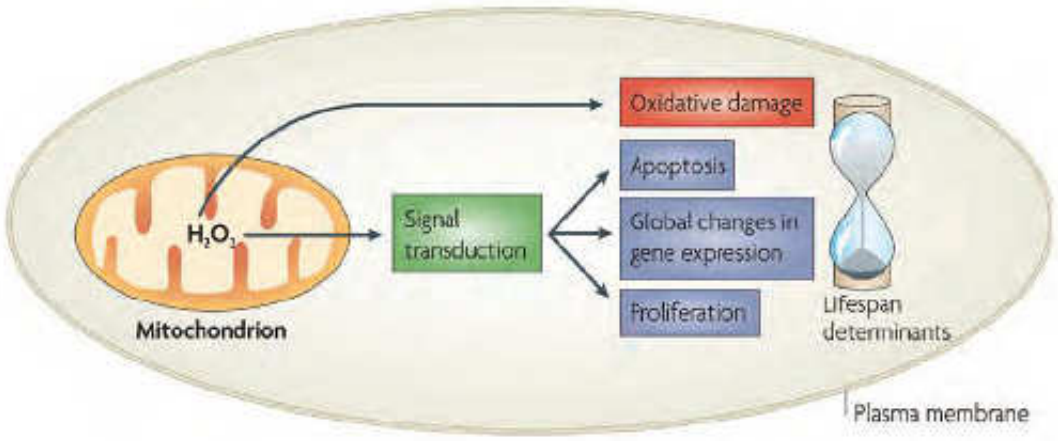

Figure 2. A model for H_2O_2 as a determinant of lifespan (Adapted with permission from Nature Publishing Group).

transduction proteins that are involved in mitogenesis or angiogenesis.

ROS have also been implicated in apoptosis (34) and cellular senescence (41). Whereas the ability of ROS to trigger senescence is thought to be secondary to their ability to induce hyperproliferation and the activation of a p53-dependent checkpoint, H_2O_2 itself is instead a direct and potent inducer of apoptosis (42). The dual character of H_2O_2 as a mediator of growth and apoptosis suggests specificity in its biological activity. Specificity could be determined by the intensity of the pro-oxidant challenge or the intracellular site of production, the latter of which further increases the complexity of redox signalling. Treatment of different primary cells with increasing doses of exogenous H_2O_2 induces proliferation, senescence or apoptosis. As H_2O_2 is generated by many compartmentalized enzymes, local variations in the concentration of H_2O_2 could also be crucial for the activation of specific targets (33).

In regard to H_2O_2 scavenging, overexpression of catalase or GPX together with SOD has been demonstrated to increase oxidative-stress resistance and lifespan in some transgenic models of *C. elegans* and *D. melanogaster* (43). Remarkably, transgenic mice that overexpress catalase in mitochondria show a specifically increased scavenging activity in mitochondria and a prolonged lifespan (44). Likewise, deletion of *p66Shc* in mice results in the decreased formation of mitochondrial H_2O_2, which correlates with delayed aging (45,46), a reduced incidence of aging-associated degenerative diseases (46-49) and increased lifespan (49).

Aging is a multifactorial process: protein turnover is impaired, telomeres shorten, somatic mutations increase and chromatin modifications (as well as global changes in gene expression) become progressively evident (51). There is no evidence that a single factor dominates during the aging process, and it seems that the different factors each contribute, perhaps in varying combinations. This may explain the enormous variability of the aging phenotype and of individual lifespan in the same species, even when genotypes and environment are controlled.

A crucial question remains unanswered: are various molecular mechanisms of aging triggered by common initiating events? H_2O_2 by itself is a powerful inducer of all of the above-mentioned aging mechanisms (Fig. 2), which suggests that it may represent a key mediator of aging. Rates of mitochondrial H_2O_2 generation are directly related to the basal metabolic rate and inversely related to the maximum lifespan of different mammalian species, which is consistent with the oxidative-stress hypothesis of aging (51). Moreover, reverse genetic models have demonstrated that altering intracellular levels of H_2O_2 modulates lifespan.

Klotho, an Anti-Aging Hormone

Over the past 15 years it has become clear that mutations in genes that regulate endocrine signalling pathways can prolong lifespan. Lifespan can be increased by altered endocrine signalling in a group of cells or a single tissue, which indicates that crosstalk between tissues functions to coordinate aging of the organism (52).

In addition to insulin and IGF1, one potential mediator of crosstalk between tissues in mammals is KLOTHO, a transmembrane protein with homology to β-glycosidases. Although KLOTHO lacks a clear antecedent in lower

metazoans (53), recent work has shown that *Klotho* overexpression in a subset of tissues is sufficient to prolong lifespan (54). A fragment of the KLOTHO protein circulates in the blood, which suggests that it might act as a hormone.

The *Klotho* gene was identified serendipitously through a hypomorphic allele that results in severe early degenerative changes and short lifespan (53). The homozygous mutant animals develop normally until 3 weeks of age, then exhibit severe growth retardation, osteoporosis, ectopic calcification, arteriosclerosis, emphysema and atrophy of the skin, thymus, testes and ovaries, and die at an average age of 61 days.

Overexpression of Klotho inhibits both insulin and IGF1 signalling and prolongs lifespan. Whether KLOTHO inhibits aging directly in all target tissues or acts indirectly through a cell non-autonomous mechanism (for example, by inhibiting insulin signalling in fat tissue) is not known (52).

The extracellular domain of KLOTHO is detectable in the blood and cerebrospinal fluid of mice and humans, which suggests that KLOTHO could function as a hormone (54,55). Transgenic overexpression of *Klotho* in the brain and testes is sufficient for rescue of the growth defect and accelerated aging phenotypes of *Klotho*-deficient mice, further supporting the hypothesis that KLOTHO can function as a humoral factor (53,54).

Consistent with the hypothesis that KLOTHO prolongs lifespan at least in part by inhibiting insulin/IGF1 signalling, heterozygous deletion of IRS1 partially rescued the reduced lifespan of male *Klotho* deficient animals and prevented the morphological changes that were reminiscent of aging (54).

KLOTHO might be an anti-aging hormone that functions by modulating insulin/IGF1 signalling and the activity of FGF23 on its receptors. In addition to these hormonal modulators, cell non-autonomous aspects of several aging pathways suggest that other endocrine regulators of aging remain to be identified (52).

DNA Damage and Aging

It is widely accepted that aging is the consequence of stochastic damage accumulation (10). Aging is unique in that it does not seem to be subject to evolutionary selection, as it occurs after the reproductive phase, suggesting that it may occur by default (57). Nevertheless, it is apparent from studies in many systems that aging is subject to regulation by evolutionarily highly conserved molecular pathways (58-60). As such, damage drives functional decline with advancing age; however, the existence of universal mechanisms that are able to promote longevity may set the pace on how rapidly damage builds up and function is lost.

Within the complex chemical machinery of each cell, all biomolecules (proteins, lipids and nucleic acids) are subject to indiscriminate damage caused by spontaneous reactions (mostly hydrolysis) and by numerous endogenous and exogenous reactive agents. It is therefore plausible that damage to multiple cellular constituents accounts for aging (10). However, damage to certain macromolecules may play a more prominent part than damage to others. The almost exclusive link between an extending class of syndromes with phenotypes resembling accelerated aging in many, but not all, organs and tissues (segmental progeria), and inborn defects in DNA metabolism points to genomic damage as a major culprit in the aging process.

In spite of its enormous length and explicit physicochemical vulnerability, cellular function relies on the integrity of the somatic genome, which must be preserved during the entire lifetime of an organism. This is why nature has invested heavily in an intricate genome maintenance apparatus, consisting of several sophisticated DNA damage repair, tolerance and checkpoint systems, as well as effector machinery that enables cell survival or triggers senescence or cell death when DNA is damaged. This elaborate network also includes intricate machineries to maintain telomeres (the ends of chromosomes), systems to repair mitochondrial DNA and as yet largely unexplored processes that maintain the epigenetic code. These mechanisms ensure that genetic information remains functionally intact for extended periods and is faithfully transmitted. Besides exogenous sources of DNA damage, such as UV and ionizing radiation, and numerous chemicals, there are also inescapable enemies from within. The culprit is the organism's own metabolism, which generates reactive oxygen species (ROS), including superoxide anions, hydrogen peroxide and hydroxyl radicals and their numerous subsequent reaction products: lipid peroxidation products, oestrogen metabolites, reactive carbonyl species, endogenous alkylating agents, spontaneous hydrolysis and deamination products (60).

Cells respond through a battery of DNA repair and genome surveillance systems that counteract DNA damage, thereby ensuring that their vital genetic information is preserved and faithfully transmitted to progeny. Nevertheless, a fraction of the damage escapes repair and accumulates, resulting in mutations, senescence or cell death and cellular dysfunction. Too much persisting

DNA damage interferes with normal DNA metabolism, such as transcription, and triggers suppression of the growth hormone/IGF1 somatotropic axis, which is known to decline with age. Dampening of the insulin/IGF1 pathway and oxidative metabolism is thought to reduce the induction and effects of DNA damage by shifting the energy equilibrium from growth and proliferation to pathways that preserve somatic maintenance and thus attempt to extend lifespan (survival response) (61).

In humans, several defective DNA-repair pathways can cause accelerated aging (progeroid) syndromes. On the other hand, certain mouse strains with defective DNA-repair systems accumulate high levels of DNA damage and yet have a normal lifespan (7). Similarly, a reduction in SOD levels in mice leads to increased oxidative DNA damage but does not affect the aging process (62). Recent work suggests that certain types of DNA damage can significantly alter the gene-expression profile of an organ and that these changes — rather than DNA damage directly — might be the cause of organ decline and aging (63). Little is known about how defective DNA repair or increased oxidative stress may cause such global gene expression changes, and more work is needed to fully understand the role of DNA damage in aging.

Despite convincing evidence for DNA damage as a trigger of transcriptional changes (63,64), it is conceivable that a change in the transcription status of a gene determines its susceptibility to DNA damage. A comprehensive (computational) analysis or the genomewide mapping of sites of DNA damage and localization of chromatin-remodelling enzymes may shed light on the complex interplay between transcriptional activity and DNA damage. Several findings suggest that DNA damage is a main trigger of nuclear aging, supporting the free-radical theory of aging (2).

Genomic Instability and Aging

Chromosomes are arguably the most difficult structures a cell has to maintain over a lifetime. The DNA in each chromosome experiences thousands of chemical alterations and DNA breaks in a single day, and the information each encodes requires strict regulation to maintain cellular identity and function. To manage these tasks, eukaryotes have evolved a complex packaging system known as chromatin, in which DNA is wrapped around a protein core of four different histone dimers and forms a nucleosome, the basic building block of chromatin. Recent studies have indicated that chromatin is a highly dynamic form of nuclear organization that influences DNA stability and gene expression patterns (65,66).

In the late 1990s, a few researchers proposed that changes in chromatin organization underlie aging-related changes in gene expression and the aging process (67,68). Changes in gene expression were already known to contribute to cellular senescence (69), a possible cause of aging, and may provide an explanation for the age-related decline in organ and tissue function in complex organisms.

Recently, a growing appreciation for the importance of chromatin in regulating gene expression and maintaining genomic integrity in complex organisms has reinvigorated interest in the link between chromatin alterations and aging. In the past 10 years, advances in nuclear imaging technologies have revealed a high level of chromatin organization that is known as the nuclear architecture (71).

The long-term maintenance of the nuclear architecture is vital for the normal functioning of cells and tissues over a lifetime. The dramatic effect of a disturbed nuclear architecture is exemplified by Hutchinson–Gilford progeria syndrome (HGPS), in which a mutation that disrupts the nuclear architecture leads to a disease with symptoms that resemble aspects of normal human aging, such as loss of hair, restricted joint mobility and atherosclerosis (72). Even cells from normal individuals undergo significant nuclear architecture changes in response to stress (73), and there are early hints that normal human aging is associated with alterations in nuclear architecture (74).

Sinclair propose that a conserved DNA-damage response induces cumulative changes in chromatin structure and nuclear architecture that are important driving forces behind the inexorable changes that occur in organisms over time. These changes include a decline in genomic integrity, alterations in gene transcription and a loss of vitality — the series of changes we commonly refer to as aging (71).

Epigenetic Balance Hypothesis

Aging results from accumulation of unrepaired cellular and molecular damage through evolved limitations in somatic maintenance and repair functions. Such damage accumulates throughout life (from the time when somatic cells and tissue first begin to form).

Longevity is controlled primarily through genes that regulate the levels of somatic maintenance and repair

functions. Note that there is no necessary assumption that these genes switch to lower levels of maintenance and repair (which would smack of programmed aging); rather, it is the set point of the genes that determines the rate at which damage accumulates (10).

In mammals, aging has been associated with large-scale changes in both nuclear architecture and chromatin structure. How might these changes contribute to the aging process? Because numerous genes are either directly or indirectly regulated by (nearby) heterochromatic regions, it is possible that changes in the epigenetic make-up of a cell might alter its gene-expression patterns, thereby changing its genomic identity (66).

Numerous epigenetic changes in nuclear architecture and gene expression have been associated with aging. More than a decade ago, Imai and colleagues showed that collagenase, a gene associated with cellular aging, is differentially regulated during cellular senescence — a phenomenon that is often referred to as cellular aging (75). This effect appears to be due to changes in the subnuclear localization of the collagenase gene as cells undergo senescence.

As mentioned earlier, the formation of transient heterochromatic foci around sites of DNA damage may explain how DNA damage might directly mediate gene repression (73). Consistent with this notion, many of the gene-expression changes that are observed in aged individuals occur in a stochastic fashion, as does most DNA damage (64, 76). It is also conceivable that certain genomic regions are more prone to damage than others, which could explain some of the predictable, co-regulated changes that are observed between aged individuals of the same species.

The 'epigenetic balance hypothesis' proposed that age-related gene-expression changes are manifestations of the redistribution of chromatin modifiers from one genomic locus to another. The model also encompasses the idea that DNA damage mediates chromatin remodelling and changes in nuclear architecture that occur over a lifetime, which fits with evidence that oxidative stress and DNA damage can accelerate the aging process (71).

A redistribution of chromatin modifiers is a natural, protective response to DNA damage, but may lead to epigenetic changes that affect genomic integrity and, thereby (at least in part), account for changes in gene expression that appear to be a hallmark of the aging process. Although this epigenetic balance hypothesis presents an appealing explanation of what we currently know about age-related changes in nuclear architecture and gene expression, it is certainly not the only way to explain the observed effects of aging.

Cellular Senescence and Aging

After a finite number of divisions, primary cell cultures enter a state of replicative senescence in which they are growth-arrested and refractory to further mitogenic stimulation. Although the relevance of in vitro senescence to organismal aging remains controversial, several studies indicate that oxidants are important in the development of the senescent phenotype. Early studies with human diploid fibroblasts revealed that cells grown in low oxygen tension exhibit a prolonged life span (77). In contrast, cells grown in the presence of high oxygen concentrations have a reduced life span and show an accelerated rate of telomere shortening per population doubling (78). Similarly, treatment of cultures of primary fibroblasts with moderate, non-lethal doses of exogenous hydrogen peroxide activates a rapid, senescence-like growth arrest (79).

What is the relationship between cellular senescence and aging? The evidence that cellular senescence actually plays a role in aging is correlative: senescent cells accumulate in vivo in mammals with increasing age and at sites of pathology (80), and many mouse and human models of premature aging are accompanied by premature cellular senescence in vitro

DNA damage and telomere dysfunction appear to activate the classical tumor-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 (81-83). The notion that senescence prevents cancer is well-supported and is not controversial (84,85). The expression of markers of senescence such as senescence-associated β-galactosidase and p16INK4a markedly increases with aging in many tissues from disparate mammalian species (85). Caloric restriction (CR) potently slows aging 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 $p16^{INK4a}$ (86-88).

Provocatively, CR, similar to $p16^{INK4a}$ deficiency (89), enhances stem-cell function with aging (90), which suggests the possibility that CR may slow aging in mammals by decreasing the activation of senescence in self-renewing compartments.

Various lines of evidence suggest that p53 plays opposing roles in the aging process. While p53 suppresses the onset of malignancy and thereby extends life span, at the same time it promotes cellular senescence and apoptosis in response to DNA damage, potentially contributing to the

clinical changes of aging. Thus, p53 function may display antagonistic pleiotropy (91).

The senescent state appears to be a universal process that is a reaction of mammalian cells to certain kinds of damage, including telomere shortening. Cellular senescence presents a puzzle in terms of evolutionary biology. The kinds of damage that cause cells to enter senescent state are very similar to those types of damage that cause other cells to enter apoptosis. From the point of view of organism and the genome, making cells undergo apoptosis makes sense because the damage cells and its progeny, carrying potentially damaged copies of the genome, are removed from the body. One may consider cells to be very cheap in terms of the overall economy of the body – millions of cells are born and die every day and there would seem to be no reason why cells should be preserved by means of the cellular senescence process, rather than killed off by means of apoptosis (92).

Cancer and Aging

In accordance with the evolutionary theory of the 'disposable soma' (93), anti-aging and anti-cancer mechanisms have adapted their respective strengths to the natural lifespan of each species and, in this manner, these mechanisms ensure that most individuals are aging-free and cancer-free for as long as they are useful or beneficial to their species. By comparing short-lived mammals, such as mice, with long-lived ones, such as humans, it becomes clear that humans must have more stringent cancer protection and anti-aging mechanisms than mice. It is therefore important that anti-aging and anti-cancer mechanisms must evolve in parallel and accommodate the natural lifespan of the species.

Cancer and aging are both fuelled by the accumulation of cellular damage. Consequently, those mechanisms that protect cells from damage simultaneously provide protection against cancer and aging. By contrast, cancer and longevity require a durable cell proliferation potential and, therefore, those mechanisms that limit indefinite proliferation provide cancer protection but favour aging. The overall balance between these convergent and divergent mechanisms guarantees fitness and a cancer-free life until late adulthood for most individuals (94).

The co-evolution of cancer and aging protection seems to have deeper roots than just the parallel adaptation of two independent processes. Indeed, recent research has unveiled convergent mechanisms that simultaneously provide cancer resistance and aging resistance, thus coupling their co-evolution. These convergent mechanisms act on common causes of cancer and aging, most notably on the generation and accumulation of cellular damage. It is well established that cellular damage is at the origin of both cancer and aging. Accordingly, those mechanisms that prevent cellular damage impinge on these two processes and provide anti-cancer and anti-aging protection. Among these mechanisms are those that improve the efficiency of energy consumption, therefore decreasing the generation of reactive oxygen species (ROS), which are considered to be a main source of endogenous damage. In addition, p53 is a master sensor of damage that triggers repair and defence responses. As discussed below, there is evidence indicating that those mechanisms or interventions that decrease ROS or improve p53 activity converge in providing protection against cancer and aging (94).

However, other 'divergent' mechanisms have also been discovered that have opposing effects on cancer and aging; specifically, protecting from cancer but promoting aging. These mechanisms include telomere shortening and the derepression of the INK4a/ARF locus. Their purpose is to prevent excessive cellular proliferation, and this produces conflicting effects on cancer and aging: while cancer protection benefits from these safeguard mechanisms, long-term regeneration and longevity become limited. As we argue below, divergent mechanisms could be mainly designed to prevent cancer, rather than to promote aging (94).

Current data indicate that senescent cells can be efficiently cleared from the organism and, therefore, it should not be assumed that senescence inducing mechanisms are necessarily pro-aging. Along these lines, apoptosis-inducing mechanisms may or may not be deleterious for the organism. Conceivably, elimination of damaged cells, either by senescence or apoptosis, can be anti-aging or pro-aging depending on the magnitude of these responses and the regeneration capacity of the damaged tissue (95).

There is a general consensus that the accumulation of cellular damage is the initiating event of both cancer and aging. Tumorigenesis is fuelled by the accumulation of genetic and epigenetic damage. Similarly, aging occurs, at least in part, because of the accumulation of macromolecular damage, which initially affects cellular proteins, lipids and DNA, but eventually impairs tissue regeneration. According to this, those mechanisms that protect cells from damage could, in principle, protect from cancer and from aging simultaneously. In this regard, it is important to keep in mind the general observation that long-lived organisms are, in general terms, more resistant to stress (96).

A common endpoint for these major tumor suppressor mechanisms is senescence. This specialized form of terminal differentiation is induced by a variety of stimuli including alterations of telomere length and structure, some forms of DNA damage (for example, oxidative stress), and activation of certain oncogenes (98,99). Senescence requires activation of the Rb and/or p53 protein, and expression of their regulators such as $p16^{INK4a}$, p21, and ARF (81-83). An important form of senescence is induced by p53, which has several antiproliferative activities including stimulation of the expression of p21, a cyclin-dependent kinase inhibitor (CDKI). CDKIs inhibit progression through the cell cycle by inhibiting cyclin-dependent kinases that phosphorylate and thereby inactivate Rb and related proteins p107 and p130 (11). The activity of p53 is predominantly mediated by inhibiting its murine double minute 2 protein (MDM2)–mediated degradation, and p53 is stabilized by diverse stimuli including DNA damage signals (e.g., resulting from oxidative stress or telomeric shortening) and oncogene activation (102-104).

The stabilization of p53 by oncogenes is in part mediated by ARF (also designated $p19^{ARF}$ in the mouse), which is induced by inappropriate cell cycle entry (105-107), and binds to MDM2, thereby inhibiting the destruction of p53 (105,108-110). Another CDKI, $p16^{INK4a}$ increases markedly in senescent cells, and correlates with increasing Rb hypophosphorylation during this process (82,83). The regulation of $p16^{INK4a}$ in senescence is not as well understood as that of p53, although it appears to be induced by several stimuli including MAP kinase signaling, oncogene activation, and growth in culture (113,114). Either p53-p21 or $p16^{INK4a}$ are able to produce Rb hypophosphorylation and initiate senescence. Some senescence-inducing stimuli (e.g., activation of the *RAS* oncogene) appear to induce both pathways, while others (e.g., DNA damage) appear to preferentially activate one or the other (97).

Damaged DNA activates checkpoint responses that are mediated by the p53 and p16-Rb pathways and that result in apoptosis or cellular senescence. If these events occur in stem/progenitor cells, tissue homeostasis is altered — a phenomenon that might contribute to aging. If, instead, DNA mutations that inactivate these checkpoint pathways accumulate, then cancer can arise (115).

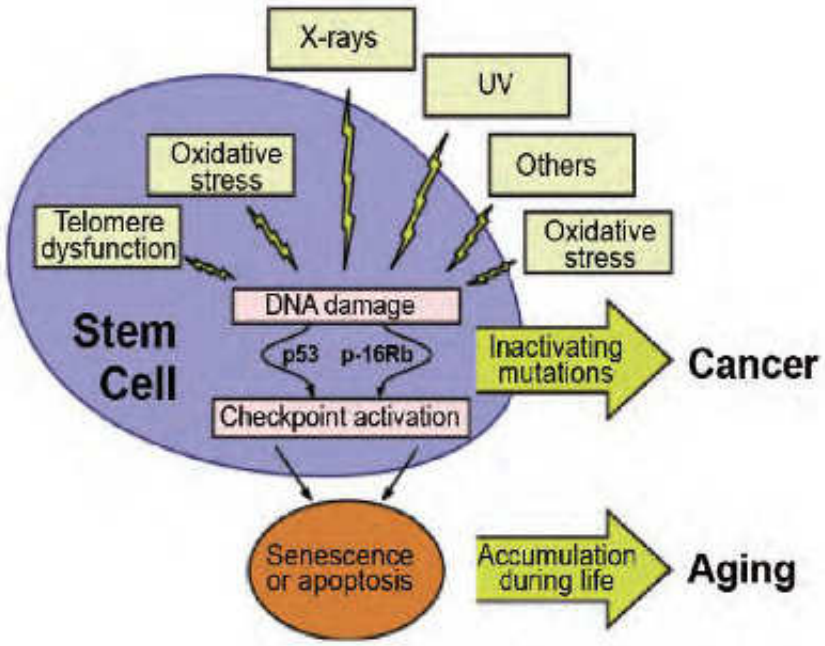

Figure 3. DNA damage accumulates as the consequence of endogenous (telomere dysfunction, oxidative stress) or exogenous (oxidative stress, γ-irradiation, UV light, and others) attacks (Adapted with permission from The American Society for Clinical Investigation).

Telomere Shortening and Stem Cell Aging

Telomeres form high-order chromatin structures that cap the ends of eukaryotic chromosomes. They contain thousands of double-stranded repeats (TTAGGG) and terminate with a single-stranded 3'-extension, which, together with specific telomeric proteins, participates in the formation of the terminal loop structure (telomere cap). Telomeres are synthesized by telomerase, a cellular reverse transcriptase that adds TTAGGG repeats onto preexisting telomeres. In cells that do not express telomerase, TTAGGG repeats are lost at each cell division, and, when telomeres reach a critical length, a checkpoint is triggered that drives cells into a metabolically active state of irreversible growth arrest, termed replicative senescence. Therefore, telomere shortening has generally been regarded as a counting mechanism that limits the mitotic potential of any cell type. In this view, cellular senescence can be considered a potent tumor-protection mechanism (115).

Cellular senescence, however, might also contribute to a decline in tissue homeostasis by exhausting the supply of progenitors or stem cells, which suggests that organism aging is the trade-off of the evolved adaptation to tumor suppression (antagonistic pleiotropy). As expected from their high replicative potential, stem cells express telomerase.

Recent findings shed new light on the molecular pathways associated with the execution of the cellular senescence program and suggest that the tumor-suppressive mechanisms involved may directly contribute to organismal aging, possibly acting at the level of stem/progenitor cells (14,97,116,117).

Telomerase is expressed in a restricted subset of normal cells: germ cells, stem/progenitor cells, and proliferating lymphocytes. Stem/progenitor cells have a long lifespan, and the machinery for self-renewal is already activated. Therefore, as compared with differentiated cells, they have greater risks of accumulating mutations and may require fewer events to sustain uncontrolled growth (115).

Mammalian aging occurs in part because of a decline in the restorative capacity of tissue stem cells. These self-renewing cells are rendered malignant by a small number of oncogenic mutations, and overlapping tumor suppressor mechanisms (e.g., $p16^{INK4a}$-Rb, ARF-p53, and the telomere) have evolved to ward against this possibility. These beneficial antitumor pathways, however, appear also to limit the stem cell life span, thereby contributing to aging (41).

Cell-intrinsic checkpoints limit the proliferative capacity of primary cells in response to telomere dysfunction. It is not known, however, whether telomere dysfunction contributes to cell-extrinsic alterations that impair stem cell function and organ homeostasis.

The dysfunctional environment limited the engraftment of transplanted wild-type hematopoietic stem cells (HSCs). Dysfunction of the hematopoietic environment was age dependent and correlated with progressive telomere shortening in bone marrow stromal cells. Telomere dysfunction impaired mesenchymal progenitor cell function, reduced the capacity of bone marrow stromal cells to maintain functional HSCs, and increased the expression of various cytokines, including granulocyte colony-stimulating factor (G-CSF), in the plasma of aging mice (118).

The accumulation of DNA damage has been associated with aging and stem cell decline (119). Telomere shortening leads to the induction of DNA damage signaling when capping function is lost at chromosome ends (120,121); such shortening occurs in most human tissues during aging and is accelerated in response to chronic diseases (122). Telomerase gene mutations limit telomere maintenance and organ homeostasis, and reduce lifespan in mice and humans (123-125).

The role for telomerase in the HSC is to partially counter the rate of telomere shortening during division of HSCs, thereby preventing premature loss of telomere function and providing added replicative capacity (126).

Stem Cell Hypothesis of Aging

With age, there is a gradual decline in the regenerative properties of most tissues due to a combination of age-dependent changes in tissue-specific stem cells and in the environmental cues that promote those cells to participate in tissue maintenance and repair (127).

These rare and specialized adult stem cells are required for tissue replacement throughout the human lifespan, and appear to be characterized by a few specific physiological and biochemical properties, particularly the capacity for self renewal (97,128,129).

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. 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 (130,131). 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) (132).

Self-renewal comes with some danger for the organism; in particular, a risk of malignant transformation (97,133,134). Unrepaired genetic lesions in stem cells are passed on to their self-renewing daughters and accumulate with aging 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 (97,128,129). 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 tumor-suppressor mechanisms may inadvertently contribute to aging by causing stem-cell arrest or attrition.

According to 'cancer–aging hypothesis', cells within a tissue are compromised by these anti-cancer mechanisms, that growth inhibitory molecules such as the cyclin-dependent kinase inhibitor $p16^{INK4a}$ and the tumor suppressor p53 exert their pro-aging effects in part through their activation in specific self-renewing compartments such as tissue-specific stem cells. 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 tumor-suppressor mechanisms in self-renewing compartments contributes to the aging processes in humans (9).

The hematopoietic system would be our choice source of stem cells to examine the effects of aging. The hematopoietic system is the best characterized of any stem cell-driven organ and, given the massive output throughout life, its degeneration is probably a major limiting factor to longevity (135).

Numerous studies have shown that aging alters HSC function with regard to mobilization (136), homing (132,136-139) and lineage choice (132,138,139). 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 (132). Therefore, the preponderance of evidence suggests that HSCs undergo cell-intrinsic aging, although there is also emerging evidence that the aging HSC microenvironment may influence HSC function in an extrinsic manner.

The most clinically significant aspect of age-dependent hematopoietic dysfunction, however, is the dramatically increased incidence of leukemias and other hematological diseases that accompany aging. Interestingly, whereas pediatric leukemias predominantly involve lymphoid lineages, the leukemias that manifest in old age are largely myeloid in origin, suggesting that the malignant capacity of different hematopoietic progenitors changes with age (132).

Hematopoietic stem cells from early-aging mice expressing a mutant p53 allele reveal that aging of stem cells can be uncoupled from aging at an organismal level. These studies show that hematopoietic stem cells are not protected from aging. Instead, loss of epigenetic regulation at the chromatin level may drive both functional attenuation of cells, as well as other manifestations of aging, including the increased propensity for neoplastic transformation (140).

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(141), MSH2 (142) or ERCC1 (143). 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 (144).

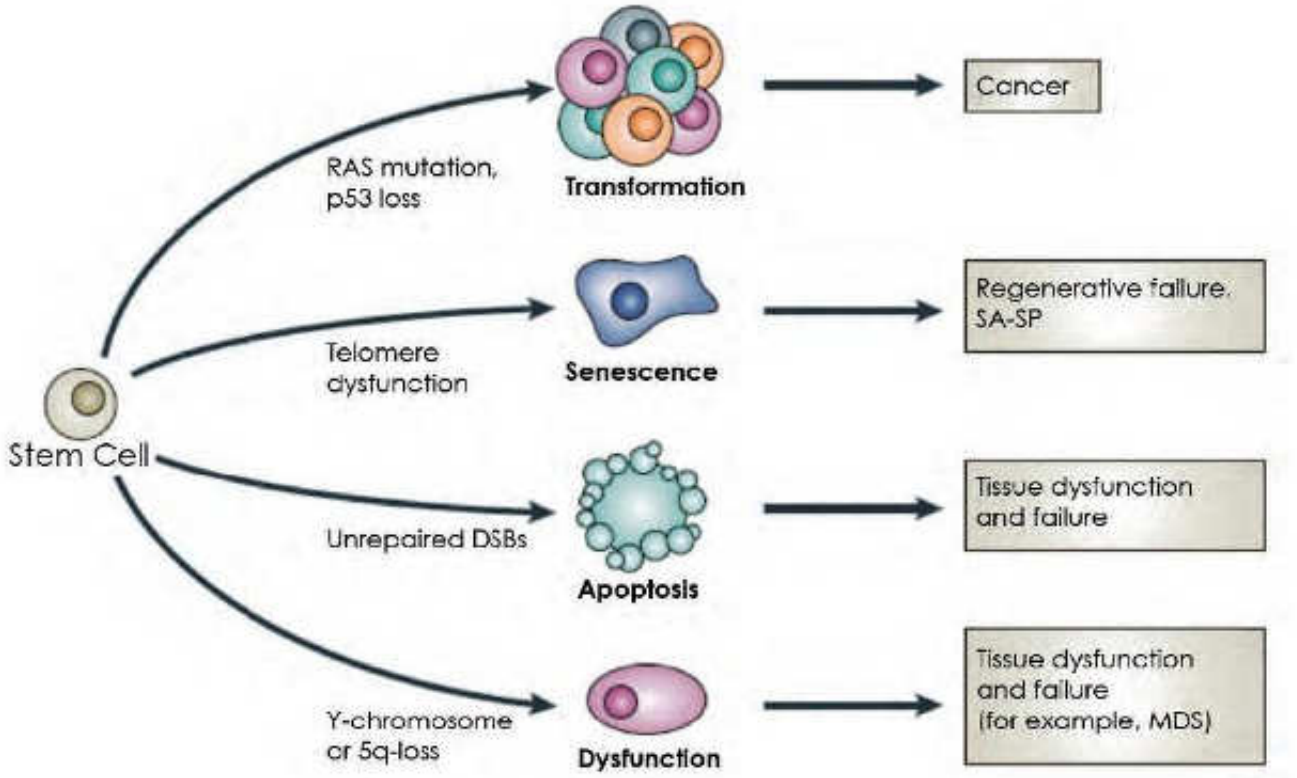

Figure 4. Fates of damaged stem cells (Adapted with permission from Nature Publishing Group).

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 (9).

In the 'DNA-damage accrual' model of aging, unrepaired (or improperly repaired) genomic damage accumulates with aging 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. 4). 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.

Related observations have likewise suggested a proaging role for p53 and its effectors in mice (145-147) and humans (148). 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.

Additionally, HSCs from $p21^{CIP}$-deficient mice demonstrate premature exhaustion (149), 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-aging and antiaging depending on the nature and duration of the stress behind its activation.

As stem cells are essential for tissue homeostasis and repair throughout life, three groups (5,140,144) have explored what factors influence alterations in their function with age. These studies, together with previous work, suggest that the story is complex, involving interactions between different networks and at several levels. At the genomic level, both internal and environmental factors cause alterations in individual genes, groups of genes through epigenetic changes, and chromosomes, at least some of which arise from direct damage to DNA. At the levels of cells and tissues, functional changes in stem cells and other cells in the tissue influence each other and are, in turn, influenced by systemic changes that may be conveyed from one tissue to another via the circulation. All of these may contribute to the possible development of cancer

in tissues throughout the body. The ultimate outcome is organismal aging.

The extent to which the effects of aging 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 (150). For the liver, it seems that differentiated hepatocytes can proliferate sufficiently to mediate effective tissue remodelling, repair and replacement normally (151), whereas stem cells might be recruited in the setting of severe injury (152). 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 (153) and heart (154) for therapeutic purposes, for example, there is limited endogenous repair capacity of these tissues following acute injuries.

Clearly, tissue – specific stem cells are responsible for the restorative aspect of these tissue dynamics. As loss of tissue function is one of the hallmarks of aging, it may be that a loss of this "maintenance" function of resident progenitor cells is tantamount to tissue aging. Understanding the environmental cues that instruct resident stem cells to participate in the normal maintenance function may suggest interventions that would slow the age – related decline in tissue structure and function by enhancing the ability of resident stem cells to maintain the youthful phenotype of the tissue. To the extend that there are irreversible biochemical changes with age, the gradual loss of tissue function may be inevitable but may be slowed, thereby to achieve the goal of healthy aging.

Conclusion

A diminished capacity to maintain tissue homeostasis is a central physiological characteristic of aging. As stem cells regulate tissue homeostasis, depletion of stem cell reserves and/or diminished stem cell function have been postulated to contributed to aging. Accumulated DNA damage could be a principle mechanism underlying age – dependent stem cell decline, and anti – cancer mechanisms such as senescence and apoptosis, which rely on telomere shortening and/or p53 and $p16^{INK4a}$ activation appear to promote stem cells aging, just as their failure is associated with cancer.

References:

1. Frisard M, Ravussin E. Energy metabolism and oxidative stress: impact on the metabolic syndrome and the aging process. *Endocrine* 2006; 29: 27–32.
2. Harman D. Aging: phenomena and theories. *Ann NY Acad Sci* 1998; 854: 1–7.
3. Schlessinger D, Van Zant G. Does functional depletion of stem cells drive aging? *Mech Aging Dev* 2001; 122: 1537–1553.
4. Park Y, Gerson SL. DNA repair defects in stem cell function and aging. *Annu Rev Med* 2005; 56: 495–508.
5. Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, Weissman IL. Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. *Nature* 2007; 447: 725 – 730.
6. Finkel TS, Holbrook NJ. Oxidants, oxidative stress and the biology of aging. *Nature* 2000; 408: 239 – 247.
7. Lombard DB, Lombard DB, Chua KF, Mostoslavsky R, Franco S, Gostissa M, Alt FW. DNA repair, genome stability, and aging. *Cell* 2005; 120: 497 – 512.
8. Brunet A, Rando TA. From stem to stem. Nature 2007; 449:
9. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol* 2007; 8: 703 – 713.
10. Kirkwood TBL. Understanding the odd science of aging. *Cell* 2005; 120: 437 – 447.
11. Karanjawala ZE, Lieber MR. DNA damage and aging. *Mech Aging Dev* 2004; 125: 405 – 416.
12. Medvedev ZA. An attempt at a rational classification of theories of aging. *Bio Rev Camb Philos Soc* 1990; 65: 375 – 398.
13. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science* 1996; 273: 59 – 63.
14. Ben – Porath I, Weinberg RA. When cells get stressed; an integrative view of cellular senescence. *J Clin Invest* 2004; 113: 8 – 13.
15. Von Zglinicki T. Replicative senescence and the art of counting. *Exp Gerontol* 2003; 38: 1259 – 1264.
16. Krtolica A, Campisi J. Cancer and aging: a model for the cancer promoting effects of the aging stroma. *Int J Biochem Cell Biol.* 2002; 34: 1401–1414.
17. Vijg J, Campisi J. Puzzles, promises and a cure for aging. *Nature* 2008; 454: 1065 – 1071.
18. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1957; 2: 298–300.
19. McCord JM, Fridovich I. Superoxide dismutase. An enzymatic function for erythrocuperin (hemocuperin). *J Biol Chem* 1969; 244: 6049–6055.
20. Promislow DE. DNA repair and the evolution of longevity: a critical analysis. *J Theor Biol* 1994; 170: 291 – 300.
21. Burkle A. Physiology and patophysiology of poly(ADP-ribosyl) ation. *Bioessays* 2001; 23: 795 – 806.

22. Kim S, Kaminker P, Campisi J. Telomeres, aging and cancer: in search of a happy ending. *Oncogene* 2002; 21: 503 – 511.
23. Wallace DC. Mitochondrial diseases in man and mouse. *Science* 1999; 283: 1482–1488.
24. Müller-Höcker J. Cytochrome-c-oxidase deficient cardiomyocytes in the human heart—an age-related phenomenon. A histochemical ultracytochemical study. *Am J Pathol* 1989; 134: 1167–1173.
25. Brierley EJ, Johnson MA, Lightowlers RN, James OF, Turnbull DM. Role of mitochondrial DNA mutations in human aging: implications for the central nervous system and muscle. *Ann Neurol* 1998; 43: 217–223.
26. Cottrell DA, Blakely EL, Johnson MA, Ince PG, Borthwick GM, Turnbull DM. Cytochrome c oxidase deficient cells accumulate in the hippocampus and choroid plexus with age. *Neurobiol Aging* 2000; 22: 265–272.
27. Taylor RW, Barron MJ, Borthwick GM, et al. Mitochondrial DNA mutations in human colonic crypt stem cells. *J Clin Invest* 2003; 112: 1351–1360.
28. Carrard G, Bulteau AL, Petropoulos I, Friguet B. Impairment of proteasome structure and function in aging. *Int J Biochem Cell Biol* 2002; 34: 1461–1474.
29. Soti C, Csermely P. Aging and molecular chaperones. *Exp Gerontol* 2003; 38: 1037–1040.
30. Terman A, Brunk UT. Aging as a catabolic malfunction. *Int J Biochem Cell Biol* 2004; 36: 2365–2375.
31. Kirkwood TBL, Boys RJ, Gillespie CS, Proctor CJ, Shanley DP, Wilkinson DJ. Towards an e-biology of aging: integrating theory and data. *Nat Rev Mol Cell Biol* 2003; 4: 243–249.
32. Kowald A, Kirkwood TBL. A network theory of aging: the interactions of defective mitochondria, aberrant proteins, free radicals and scavengers in the aging process. *Mutat Res* 1996; 316: 209–236.
33. Giorgio M, Trinei M, Migliaccio E, Pelici PG. Hydrogen peroxide: a metabolic by-product or a common mediator of aging signals? *Nat Rev Mol Cell Biol* 2007; 8: 722 – 728.
34. Stone JR, Yang S. Hydrogen peroxide: a signaling messenger. *Antioxid redox Signal* 2006; 8: 243 – 270.
35. Finkel T. Redox – dependent signal transduction. *Febs Lett* 2000; 476: 52 – 54.
36. Alexandrova AY, Kopnin PB, Vasiliev JM, Kopnin BP. ROS up – regulation mediates Ras – induced changes of cell morphology and motility. *Exp Cell Res* 2006; 312: 2066 – 2073.
37. Vafa O, et al. c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability. *Mol Cell* 2002; 9: 1031–1044.
38. Schimmel M, Bauer G. Proapoptotic and redox state-related signaling of reactive oxygen species generated by transformed fibroblasts. *Oncogene* 2002; 21: 5886–5896.
39. Giles G I. The redox regulation of thiol dependent signaling pathways in cancer. *Curr Pharm Des* 2006; 12: 4427–4443.
40. North S, Moenner M, Bikfalvi A. Recent developments in the regulation of the angiogenic switch by cellular stress factors in tumors. *Cancer Lett* 2005; 218: 1–14.
41. Passos JF, Von Zglinicki,T. Oxygen free radicals in cell senescence: are they signal transducers? *Free Radic Res* 2006; 40: 1277–1283.
42. Kowaltowski AJ, Castilho RF, Vercesi AE. Mitochondrial permeability transition and oxidative stress. *FEBS Lett* 2001; 495: 12–15.
43. Landis GN, Tower,J. Superoxide dismutase evolution and life span regulation. *Mech Aging Dev* 2005; 126: 365–379.
44. Schriner SE, Linford NJ, Martin GM, et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 2005; 308: 1909–1911.
45. Francia P, delli Gatti C, Bachschmid M, et al. Deletion of p66Shc gene protects against age-related endothelial dysfunction. *Circulation* 2004; 110: 2889–2895.
46. Menini S, Amadio L, Oddi G, et al. Deletion of p66Shc longevity gene protects against experimental diabetic glomerulopathy by preventing diabetes-induced oxidative stress. *Diabetes* 2006; 55: 1642–1650.
47. Napoli C, Martin-Padura I, de Nigris F, et al. Deletion of the p66Shc longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet. *Proc Natl Acad Sci USA* 2003; 18: 2112–2116.
48. Rota M, LeCapitaine N, Hosoda T, et al. Diabetes promotes cardiac stem cell aging and heart failure, which are prevented by deletion of the p66Shc gene. *Circ Res* 2006; 99: 42–52.
49. Migliaccio E, Giorgio M, Mele S, et al. The p66Shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* 1999; 402: 309–313.
50. Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine* (Oxford University Press, 1998).
51. Sohal RS, Allen RG. Oxidative stress as a causal factor in differentiation and aging: a unifying hypothesis. *Exp Gerontol* 1990; 25: 499–522.
52. Russel SJ, Kahn CR. Endocrine regulation of aging. *Nat Rev Mol Cell Biol* 2007; 8: 681 – 691.
53. Kuro-o M, Matsumura Y, Aizawa H, et al. Mutation of the mouse klotho gene leads to a syndrome resembling aging. *Nature* 1997; 390: 45–51.
54. Kurosu H, Yamamoto M, Clark JD, et al. Suppression of aging in mice by the hormone Klotho. *Science* 2005; 309: 1829–1833.
55. Imura A, Iwano A, Tohyama O, et al. Secreted Klotho protein in sera and CSF: implication for post-translational cleavage in release of Klotho protein from cell membrane. *FEBS Lett* 2004; 565: 143–147.
56. Kirkwood TB, Cremer T. Cytogerontology since 1881: a reappraisal of August Weismann and a review of modern progress. *Hum Genet* 1982; 60: 101–121.
57. Guarente L, Kenyon C. Genetic pathways that regulate aging in model organisms. *Nature* 2000; 408: 255–262.
58. Partridge L, Gems D. Mechanisms of aging: public or private? *Nat Rev Genet* 2002; 3: 165–175.
59. Kenyon C. The plasticity of aging: insights from long-lived mutants. *Cell* 2005; 120: 449–460.
60. De Bont R, van Larebeke N. Endogenous DNA damage in humans: a review of quantitative data. *Mutagenesis* 2004; 19: 169 – 185.
61. Garinis GA, van der Horst GTJ, Vijg J, Hoeijmakers HJ. DNA damage and aging: new – age ideas for an age – old problem. *Nat Cell Biol* 2008; 11: 1241 – 1247.
62. Van Remmen H, Ikeno Y, Hamilton M, et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol Genomics* 2003; 16: 29–37.
63. Niedernhofer LJ, Garinis GA, Raams A, et al. A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. *Nature* 2006; 444: 1038 – 1043.
64. Bahar R. Increased cell-to-cell variation in gene expression in aging mouse heart. *Nature* 2006; 441: 1011 – 1014.

65. Cheutin T, McNairn AJ, Jenuwein T, Gilbert DM, Singh PB, Misteli T.. Maintenance of stable heterochromatin domains by dynamic HP1 binding. *Science* 2003; 299: 721–725.

66. Grewal SI, Jia S. Heterochromatin revisited. *Nature Rev Gene* 2007; 8: 35–46.

67. Villeponteau B. The heterochromatin loss model of aging. *Exp Gerontol* 1997; 32: 383–394.

68. Imai S, Kitano H. Heterochromatin islands and their dynamic reorganization: a hypothesis for three distinctive features of cellular aging. *Exp Gerontol* 1998; 33: 555–570.

69. Goldstein, S. Replicative senescence: the human fibroblast comes of age. *Science* 1990; 249: 1129–1133.

70. Campisi, J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 2005; 120: 513–522.

71. Oberdoerffer P, Sinclair DA. The role of nuclear architecture in genomic instability and aging. *Nat Rev Mol Cell Biol* 2007; 8: 692 – 702.

72. Hennekam RC, Hutchinson–Gilford progeria syndrome: review of the phenotype. *Am J Med Genet A* 2006; 140: 2603–2624.

73. Narita, M. et al. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell* 2003; 113: 703–716.

74. Scaffidi P, Misteli T. Lamin A-dependent nuclear defects in human aging. *Science* 2006; 312: 1059–1063.

75. Imai S, Nishibayashi S, Takao K, et al. Dissociation of Oct-1 from the nuclear peripheral structure induces the cellular aging-associated collagenase gene expression. *Mol Biol Cell* 1997; 8: 2407–2419.

76. Malins DC, Anderson KM, Jaruga P, et al. Oxidative changes in the DNA of stroma and epithelium from the female breast: potential implications for breast cancer. *Cell Cycle* 2006; 5: 1629–1632.

77. Packer L, Fuehr K. Low oxygen concentration extends the lifespan of cultured human diploid cells. *Nature* 1977; 267: 423–425.

78. von Zglinicki T, Saretzki G, Docke W, Lotze C. Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? *Exp Cell Res* 1995; 220: 186–193.

79. Chen Q, Ames B N. Senescence-like growth arrest induced by hydrogen peroxide in human diploid fibroblast F65 cells. *Proc Natl Acad Sci USA* 1994; 91: 4130–4134.

80. Itahana K, Campisi J, Dimri GP. Mechanisms of cellular senescence in human and mouse cells, *Biogerontology* 2004; 5: 1–10.

81. Kamijo T, Zindy F, Roussel MF, et al. Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. *Cell* 1997; 91: 649–659.

82. Stein GH, Drullinger LF, Soulard A, Dulic V. Differential roles for cyclin-dependent kinase inhibitors p21 and p16 in the mechanisms of senescence and differentiation in human fibroblasts. *Mol Cell Biol* 1999; 19: 2109–2117.

83. Alcorta DA, Xiong Y, Phelps D, Hannon G, Beach D, Barrett JC.. Involvement of the cyclindependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. *Proc Natl Acad Sci USA* 1996; 93: 13742–13747.

84. Campisi J. Suppressing cancer: the importance of being senescent. *Science* 2005; 309: 886–887.

85. Kim WY, Sharpless NE. The regulation of INK4/ARF in cancer and aging. *Cell* 2006; 127: 265–275.

86. Sone H, Kagawa Y. Pancreatic β cell senescence contributes to the pathogenesis of type 2 diabetes in high-fat diet-induced diabetic mice. *Diabetologia* 2005; 48: 58–67.

87. Krishnamurthy, J. et al. Ink4a/Arf expression is a biomarker of aging. *J Clin Invest* 2004; 114: 1299–1307.

88. Edwards MG, Anderson RM, Yuan M, Kendziorski CM, Weindruch R, Prolla TA. Gene expression profiling of aging reveals activation of a p53-mediated transcriptional program. *BMC Genomics* 2007; 8: e80.

89. Janzen V, Forkert R, Fleming HE, et al. Stem-cell aging modified by the cyclin-dependent kinase inhibitor p16INK4a. *Nature* 2006; 443: 421–426.

90. Chen J, Astle CM, Harrison DE. Hematopoietic senescence is postponed and hematopoietic stem cell function is enhanced by dietary restriction. *Exp Hematol* 2003; 31: 1097–1103.

91. Campisi J. Between Scylla and Charybdis: p53 links tumor suppression and aging. *Mech Aging Dev* 2002; 123: 567 – 573.

92. Hornsby PJ. Cellular senescence and tissue aging in vivo. *J Gerontol A Biol Sci Med Sci*. 2002; 57A: B251 – B256.

93. Kirkwood T B, Austad SN. Why do we age? *Nature* 2000; 408: 233–238.

94. Serrano M, Blasco MA. Cancer and aging: convergent and divergent mechanisms. *Nat rev mol cell biol* 2007; 8: 715 – 721.

95. Xue W, Zender L, Miething C, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* 2007; 445: 656–660.

96. Kirkwood TL, Kapahi P, Shanley DP. Evolution, stress, and longevity. *J Anat* 2000; 197: 587–590.

97. Sharpless NE, DePinho RA. Telomeres, stem cells, senescence, and cancer. *J Clin Invest* 2004; 113: 160 – 168.

98. Wright WE, Shay JW. Historical claims and current interpretations of replicative aging. *Nat Biotechnol* 2002; 20: 682–688.

99. Campisi J. Cellular senescence as a tumor-suppressor mechanism. *Trends Cell Biol* 2001; 11: S27–S31.

100. Sage J, Miller AL, Perez-Mancera PA, Wysocki JM, Jacks T. Acute mutation of retinoblastoma gene function is sufficient for cell cycle re-entry. *Nature* 2003; 424: 223–228.

101. Classon M, Harlow E. The retinoblastoma tumour suppressor in development and cancer. *Nat Rev Cancer* 2002; 2: 910–917.

102. Vousden KH. p53: death star. *Cell* 2000; 103: 691–694.

103. Sharpless NE, DePinho RA. p53: good cop/bad cop. *Cell* 2002; 110: 9–12.

104. Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell* 1997; 88: 323–331.

105. Pomerantz J, Schreiber-Agus N, Liégeois NJ, et al. The Ink4a tumor suppressor gene product, p19Arf, interacts with MDM2 and neutralizes MDM2's inhibition of p53. *Cell* 1998; 92: 713–723.

106. Zindy F, Eischen CM, Randle DH, et al. Myc signaling via the ARF tumor suppressor regulates p53-dependent apoptosis and immortalization. *Genes Dev* 1998; 12: 2424–2433.

107. de Stanchina E, McCurrach ME, Zindy F, et al. E1A signaling to p53 involves the p19(ARF) tumor suppressor. *Genes Dev* 1998; 12: 2434–2442.

108. Zhang Y, Xiong Y, Yarbrough WG. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell* 1998; 92: 725–734.

109. Stott FJ, Bates S, James MC, et al. The alternative product from the human CDKN2A locus, p14(ARF), participates in a regulatory feedback loop with p53 and MDM2. EMBO J 1998; 17: 5001–5014.

110. Kamijo T, Weber JD, Zambetti G, Zindy F, Roussel MF, Sherr

CJ.. Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. *Proc Natl Acad Sc. USA* 1998, 95: 8292–8297.

111. Zhu J, Woods D, McMahon M, Bishop JM. Senescence of human fibroblasts induced by oncogenic Raf. *Genes Dev* 1998; 12: 2997–3007.

112. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16^{INK4a}. *Cell* 1997; 88: 593–602.

113. Zhu J, Woods D, McMahon M, Bishop JM. Senescence of human fibroblasts induced by oncogenic Raf. *Genes Dev* 1998, 12: 2997–3007.

114. Zindy F, Quelle DE, Roussel MF, Sherr;CJ. Expression of the p16INK4a tumor suppressor versus other INK4 family members during mouse development and aging. *Oncogene* 1997; 15: 203–211.

115. Pelicci PG. Do tumor – suppressive mechanisms contribute to organism aging by inducing stem cell senescence? J Clin Invest 2004, 113: 4 – 7.

116. Park IK, Morrison SJ, Clarke MF. Bmi-1, stem cells, and senescence regulation. *J Clin Invest* 2004; 113: 175 – 179.

117. Kahlem P, Dörken B, Schmitt CA. Cellular senescence in cancer treatment: friend or foe? *J Clin Invest* 2004; 113: 169 – 174.

118. Ju Z, Jiang H, Jaworsky M, et al. Telomere dysfunction induces environmental alterations limiting hemaotopoeitic stem cell function and engraftment. *Nat Med* 2007; 13: 742 – 747.

119. Lieber MR, Karanjawala ZE. Aging, repetitive genomes and DNA damage. *Nat Rev Mol Cell Biol* 2004; 5: 69–75.

120. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, et al. A DNA damage checkpoint response in telomere-initiated senescence. *Nature* 2003; 426: 194–198.

121. Vazin H, Benchimol S. From telomere loss to p53 induction and activation of a DNA-damage pathway at senescence: the telomere loss/DNA damage model of cell aging. *Exp Gerontol* 1996; 31: 295–301.

122. Djojosubroto MW, Choi YS, Lee HW, Rudolph KL. Telomeres and telomerase in aging, regeneration and cancer. *Mol Cells* 2003; 15: 164–175.

123. Vulliamy T, Marrone A, Szydlo R, Walne A, Mason PJ, Dokal I. Disease anticipation is associated with progressive telomere shortening in families with dyskeratosis congenita due to mutations in TERC. *Nat Genet* 2004; 36: 447–449.

124. Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C, DePinho RA. Longevity, stress response, and cancer in aging telomerase deficient mice. *Cell* 1999; 96: 701–712.

125. Herrera E, Samper E, Martin-Caballero J, Flores JM, Lee HW, Blasco MA. Disease states associated with telomerase deficiency appear earlier in mice with short telomeres. *EMBO J* 1999; 18: 2950–2960.

126. Allsopp RC, Morin GB, DePinho R, Harley CB, Weissman IL. Te;omerase is required to slow telomere shortening and extend replicative lifespan of HSCs during serial transplantation. *Blood* 2003; 102: 517 – 520.

127. Conboy IM, Rando TA. Aging, stem cells and tissue regeneration. *Cell Cycle* 2005; 4: 407 – 410.

128. Bryder D, Rossi DJ, Weissman IL. Hematopoietic stem cells: the paradigmatic tissue specific stem cell. *Am J Pathol* 2006; 169: 338–346.

129. Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature* 2006; 441: 1068–1074.

130. Hodgson GS, Bradley TR. In vivo kinetic status of hematopoietic stem and progenitor cells as inferred from labeling with bromodeoxyuridine. *Exp Hematol* 1984, 12: 683–687.

131. Passegue E, Wagers AJ, Giuriato S, Anderson WC, Weissman IL. Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. *J Exp Med* 2005; 202: 1599–1611.

132. Rossi DJ, Bryder D, Zahn JM, et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. *Proc NatlAcad Sci USA* 2005; 102: 9194–9199.

133. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; 414: 105–111.

134. Campisi J. Cancer and aging: rival demons? *Nature Rev Cancer* 2003; 3: 339–349.

135. Pearce DJ, Anjos – Afonso F, Ridler CM, Eddaoudi A, Bonnet D. Age – dependent increase in side population distribution within hematopoiesis: implications for our understanding the mechanisms of aging. *Stem Cells* 2007; 25: 828 – 835.

136. Xing Z, Ryan MA, Daria D. Increased hematopoietic stem cell mobilization in aged mice. *Blood* 2006; 108: 2190–2197.

137. Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL. The aging of hematopoietic stem cells. *Nature Med* 1996; 2: 1011–1016.

138. Sudo K, Ema H, Morita Y, Nakauchi H. Ageassociated characteristics of murine hematopoietic stem cells. *J Exp Med* 2000; 192: 1273–1280.

139. Liang Y, Van Zan, G, Szilvassy SJ. Effects of aging on the homing and engraftment of murine hematopoietic stem and progenitor cells. *Blood* 2005; 106: 1479–1487.

140. Chambers SM, Shaw CA, Gatza C, Fisk CJ, Donehower L, Goodell MA. Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. *Plos Biol* 2007; 5: 1750 – 1762.

141. Navarro S, Meza NW, Quintana Bustamante O, et al. Hematopoietic dysfunction in amouse model for Fanconi anemia group D1. *Mol Ther* 2006; 14: 525–535.

142. Reese JS, Liu L, Gerson SL. Repopulating defect of mismatch repair-deficient hematopoietic stem cells. *Blood* 2003; 102: 1626–1633.

143. Prasher JM, Lalai AS, Heijmans-Antonissen C, et al. Reduced hematopoietic reserves in DNA interstrand crosslink repair-deficient Ercc1–/– mice. *EMBO J* 2005; 24: 861–871.

144. Nijnik A, Woodbine L, Marchetti C, et al. DNA repair is limiting for haematopoietic stem cells during aging. *Nature* 2007; 447: 686–690.

145. Choudhury AR, Ju Z, Djojosubroto MW, et al. Cdkn1a deletion improves stem cell function and lifespan of mice with dysfunctional telomeres without accelerating cancer formation. *Nature Genet* 2007; 39: 99–105.

146. Tyner SD, Venkatachalam S, Choi J, et al. p53 mutant mice that display early aging-associated phenotypes. *Nature* 2002; 415: 45–53.

147. Maier B, Gluba W, Bernier B. Modulation of mammalian life span by the short isoform of p53. *Genes Dev* 2004; 18: 306–319.

148. Orsted DD, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. Tumor suppressor p53 Arg72Pro polymorphism and longevity, cancer survival, and risk of cancer in the general population. *J Exp Med* 2007; 204: 1295–1301.

149. Cheng T, Rodrigues N, Shen H, et al. Hematopoietic stem cell quiescence maintained by p21cip1/waf1. *Science* 2000; 287: 1804–1808.

150. Morgan JE, Partridge TA. Muscle satellite cells. *Int J Biochem*

Cell Biol 2003; 35: 1151–1156.

151. Sigal SH, Brill S, Fiorino AS, Reid LM. The liver as a stem cell and lineage system. *Am J Physiol* 1992; 263: G139–G148.
152. Oh SH, Hatch HM, Petersen BE. Hepatic oval 'stem' cell in liver regeneration. *Semin Cell Dev Biol* 2002; 13: 405–409.
153. Gage FH. Stem cells of the central nervous system. Curr Opin *Neurobiol* 1998; 8: 671–676.
154. Leri A, Kajstura J, Anversa P. Cardiac stem cells and mechanisms of myocardial regeneration. *Physiol Rev* 2005; 85: 1373–1416.