

The Hallmarks of Aging

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Aging is characterized by a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death. This deterioration is the primary risk factor for major human pathologies, including cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. Aging research has experienced an unprecedented advance over recent years, particularly with the discovery that the rate of aging is controlled, at least to some extent, by genetic pathways and biochemical processes conserved in evolution. This Review enumerates nine tentative hallmarks that represent common denominators of aging in different organisms, with special emphasis on mammalian aging. These hallmarks are: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. A major challenge is to dissect the interconnectedness between the candidate hallmarks and their relative contributions to aging, with the final goal of identifying pharmaceutical targets to improve human health during aging, with minimal side effects.

Introduction

Aging, which we broadly define as the time-dependent functional decline that affects most living organisms, has attracted curiosity and excited imagination throughout the history of humankind. However, it has only been 30 years since a new era in aging research was inaugurated following the isolation of the first long-lived strains in *Caenorhabditis elegans* (*C. elegans*) (Klass, 1983). Nowadays, aging is subjected to scientific scrutiny based on the ever-expanding knowledge of the molecular and cellular bases of life and disease. The current situation of aging research exhibits many parallels with that of cancer research in previous decades. The cancer field gained major momentum in 2000, with the publication of a landmark paper that enumerated six hallmarks of cancer (Hanahan and Weinberg, 2000), recently expanded to ten (Hanahan and Weinberg, 2011). This categorization has helped to conceptualize the essence of cancer and its underlying mechanisms.

At first sight, cancer and aging may seem to be opposite processes: cancer is the consequence of an aberrant gain of cellular fitness, whereas aging is characterized by a loss of fitness. At a deeper level, however, cancer and aging share common origins. The time-dependent accumulation of cellular damage is widely considered to be the general cause of aging (Gems and Partridge, 2013; Kirkwood, 2005; Vijg and Campisi, 2008).

Concomitantly, cellular damage may occasionally provide aberrant advantages to certain cells, which can eventually produce cancer. Therefore, cancer and aging can be regarded as two different manifestations of the same underlying process—namely, the accumulation of cellular damage. In addition, several of the pathologies associated with aging, such as atherosclerosis and inflammation, involve uncontrolled cellular overgrowth or hyperactivity (Blagosklonny, 2008). Based on this conceptual framework, several critical questions have arisen in the field of aging regarding the physiological sources of aging-causing damage, the compensatory responses that try to re-establish homeostasis, the interconnection between the different types of damage and compensatory responses, and the possibilities to intervene exogenously to delay aging.

Here, we have attempted to identify and categorize the cellular and molecular hallmarks of aging. We propose nine candidate hallmarks that are generally considered to contribute to the aging process and together determine the aging phenotype (Figure 1). Given the complexity of the issue, we have emphasized current understanding of mammalian aging while recognizing pioneer insights from simpler model organisms (Gems and Partridge, 2013; Kenyon, 2010). Each hallmark should ideally fulfill the following criteria: (1) it should manifest during normal aging; (2) its experimental aggravation should accelerate aging; and (3)

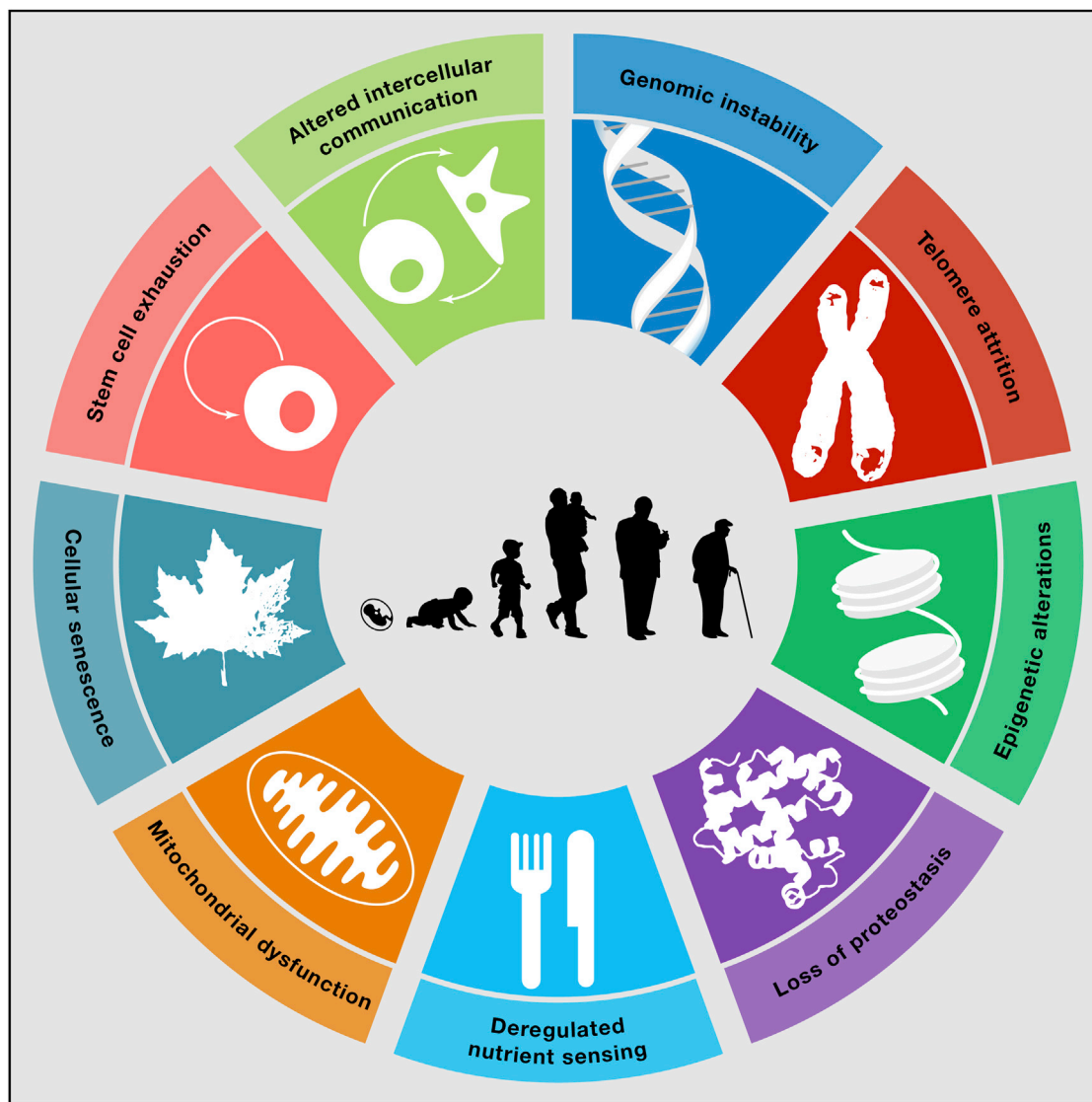


Figure 1. The Hallmarks of Aging

The scheme enumerates the nine hallmarks described in this Review: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.

its experimental amelioration should retard the normal aging process and hence increase healthy lifespan. This set of ideal requisites is met to varying degrees by the proposed hallmarks, an aspect that will be discussed in detail for each of them. The last criterion is the most difficult to achieve, even if restricted to just one aspect of aging. For this reason, not all of the hallmarks are fully supported yet by interventions that succeed in ameliorating aging. This caveat is tempered by the extensive interconnectedness between the aging hallmarks, implying that experimental amelioration of one particular hallmark may impinge on others.

Genomic Instability

One common denominator of aging is the accumulation of genetic damage throughout life (Moskalev et al., 2012)

(Figure 2A). Moreover, numerous premature aging diseases, such as Werner syndrome and Bloom syndrome, are the consequence of increased DNA damage accumulation (Burtner and Kennedy, 2010), though the relevance of these and other progeroid syndromes to normal aging remains unresolved due, in part, to the fact that they recapitulate only some aspects of aging. The integrity and stability of DNA are continuously challenged by exogenous physical, chemical, and biological agents, as well as by endogenous threats, including DNA replication errors, spontaneous hydrolytic reactions, and reactive oxygen species (ROS) (Hoeijmakers, 2009). The genetic lesions arising from extrinsic or intrinsic damages are highly diverse and include point mutations, translocations, chromosomal gains and losses, telomere shortening, and gene disruption caused by the integration of viruses or transposons. To minimize these lesions,

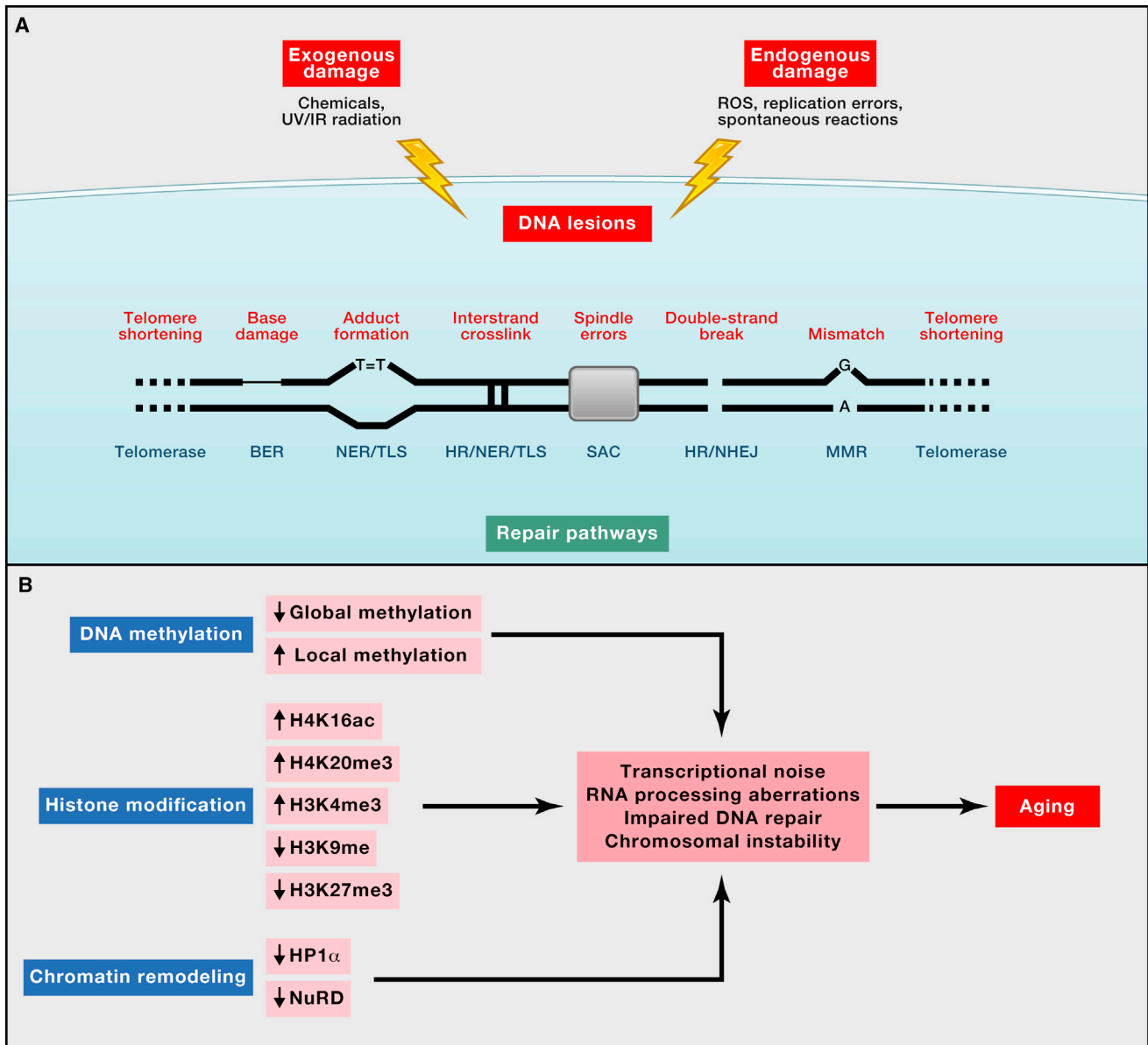


Figure 2. Genomic and Epigenetic Alterations

(A) Genomic instability and telomere attrition. Endogenous or exogenous agents can stimulate a variety of DNA lesions that are schematically represented on one single chromosome. Such lesions can be repaired by a variety of mechanisms. Excessive DNA damage or insufficient DNA repair favors the aging process. Note that both nuclear DNA and mitochondrial DNA (not represented here) are subjected to age-associated genomic alterations. BER, base excision repair; HR, homologous recombination; NER, nucleotide excision repair; NHEJ, nonhomologous end-joining; MMR, mismatch repair; ROS, reactive oxygen species; TLS, translesion synthesis; SAC, spindle assembly checkpoint (Vijg, 2007).

(B) Epigenetic alterations. Alterations in the methylation of DNA or acetylation and methylation of histones, as well as of other chromatin-associated proteins, can induce epigenetic changes that contribute to the aging process.

organisms have evolved a complex network of DNA repair mechanisms that are collectively capable of dealing with most of the damages inflicted to nuclear DNA (Lord and Ashworth, 2012). The genomic stability systems also include specific mechanisms for maintaining the appropriate length and functionality of telomeres (which are the topic of a separate hallmark; see below) and for ensuring the integrity of mitochondrial DNA (mtDNA) (Blackburn et al., 2006; Kazak et al., 2012). In addition to these

direct lesions in the DNA, defects in the nuclear architecture, known as laminopathies, can cause genome instability and result in premature aging syndromes (Worman, 2012).

Nuclear DNA

Somatic mutations accumulate within cells from aged humans and model organisms (Moskalev et al., 2012). Other forms of DNA damage, such as chromosomal aneuploidies and copy number variations, have also been found associated with aging

(Faggioli et al., 2012; Forsberg et al., 2012). Increased clonal mosaicism for large chromosomal anomalies has been also reported (Jacobs et al., 2012; Laurie et al., 2012). All of these forms of DNA alterations may affect essential genes and transcriptional pathways, resulting in dysfunctional cells that, if not eliminated by apoptosis or senescence, may jeopardize tissue and organismal homeostasis. This is especially relevant when DNA damage impacts the functional competence of stem cells, thus compromising their role in tissue renewal (Jones and Rando, 2011; Rossi et al., 2008) (see “Stem Cell Exhaustion”).

Causal evidence for the proposed links between lifelong increase in genomic damage and aging has arisen from studies in mice and humans, showing that deficiencies in DNA repair mechanisms cause accelerated aging in mice and underlie several human progeroid syndromes, such as Werner syndrome, Bloom syndrome, xeroderma pigmentosum, trichothiodystrophy, Cockayne syndrome, and Seckel syndrome (Gregg et al., 2012; Hoeijmakers, 2009; Murga et al., 2009). Moreover, transgenic mice overexpressing BubR1, a mitotic checkpoint component that ensures accurate segregation of chromosomes, exhibit an increased protection against aneuploidy and cancer, as well as extended healthy lifespan (Baker et al., 2013). The latter findings provide experimental evidence that artificial reinforcement of nuclear DNA repair mechanisms may delay aging.

Mitochondrial DNA

Mutations and deletions in aged mtDNA may also contribute to aging (Park and Larsson, 2011). mtDNA has been considered a major target for aging-associated somatic mutations due to the oxidative microenvironment of the mitochondria, the lack of protective histones in the mtDNA, and the limited efficiency of the mtDNA repair mechanisms compared to those of nuclear DNA (Linnane et al., 1989). The causal implication of mtDNA mutations in aging has been controversial because of the multiplicity of mitochondrial genomes, which allows for the coexistence of mutant and wild-type genomes within the same cell, a phenomenon that is referred to as “heteroplasmy.” However, single-cell analyses have revealed that, despite the low overall level of mtDNA mutations, the mutational load of individual aging cells becomes significant and may attain a state of homoplasmy in which one mutant genome prevails (Khrapko et al., 1999). Interestingly, contrary to previous expectations, most mtDNA mutations in adult or aged cells appear to be caused by replication errors early in life, rather than by oxidative damage. These mutations may undergo polyclonal expansion and cause respiratory chain dysfunction in different tissues (Ameur et al., 2011). Studies of accelerated aging in HIV-infected patients treated with antiretroviral drugs, which interfere with mtDNA replication, have supported the concept of clonal expansion of mtDNA mutations originated early in life (Payne et al., 2011).

The first evidence that mtDNA damage might be important for aging and age-related diseases derived from the identification of human multisystem disorders caused by mtDNA mutations that partially phenocopy aging (Wallace, 2005). Further causative evidence comes from studies on mice that are deficient in mitochondrial DNA polymerase γ . These mutant mice exhibit aspects of premature aging and reduced lifespan in association with the accumulation of random point mutations and deletions

in mtDNA (Kujoth et al., 2005; Trifunovic et al., 2004; Vermulst et al., 2008). Cells from these mice show impaired mitochondrial function, but unexpectedly, this is not accompanied by increased ROS production (Edgar et al., 2009; Hiona et al., 2010). Moreover, stem cells from these progeroid mice are particularly sensitive to the accumulation of mtDNA mutations (Ahlqvist et al., 2012) (see “Stem Cell Exhaustion”). Future studies are necessary to determine whether genetic manipulations that decrease the load of mtDNA mutations are able to extend lifespan.

Nuclear Architecture

Defects in the nuclear lamina can also cause genome instability (Dechat et al., 2008). Nuclear lamins constitute the major components of the nuclear lamina and participate in genome maintenance by providing a scaffold for tethering chromatin and protein complexes that regulate genomic stability (Gonzalez-Suarez et al., 2009; Liu et al., 2005). The nuclear lamina attracted the attention of aging researchers after the discovery that mutations in genes encoding protein components of this structure, or factors affecting their maturation and dynamics, cause accelerated aging syndromes such as the Hutchinson-Gilford and the Néstor-Guillermo progeria syndromes (HGPS and NGPS, respectively) (Cabanillas et al., 2011; De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003). Alterations of the nuclear lamina and production of an aberrant prelamin A isoform called progerin have also been detected during normal human aging (Ragnauth et al., 2010; Scaffidi and Misteli, 2006). Telomere dysfunction also promotes progerin production in normal human fibroblasts upon prolonged *in vitro* culture, suggesting intimate links between telomere maintenance and progerin expression during normal aging (Cao et al., 2011). In addition to these age-associated changes in A-type lamins, lamin B1 levels decline during cell senescence, pointing to its utility as a biomarker of this process (Freund et al., 2012; Shimi et al., 2011).

Animal and cellular models have facilitated the identification of the stress pathways elicited by aberrations in the nuclear lamina characteristic of HGPS. These pathways include the activation of p53 (Varela et al., 2005), deregulation of the somatotrophic axis (Mariño et al., 2010), and attrition of adult stem cells (Espada et al., 2008; Scaffidi and Misteli, 2008). The causal relevance of nuclear lamina abnormalities in premature aging has been supported by the observation that decreasing prelamin A or progerin levels delays the onset of progeroid features and extends lifespan in mouse models of HGPS. This can be achieved by systemic injection of antisense oligonucleotides, farnesyltransferase inhibitors, or a combination of statins and aminobisphosphonates (Osorio et al., 2011; Varela et al., 2008; Yang et al., 2006). Restoration of the somatotrophic axis through hormonal treatments or inhibition of NF- κ B signaling also extends lifespan in these progeroid mice (Mariño et al., 2010; Osorio et al., 2012). Moreover, a homologous recombination-based strategy has been developed to correct the *LMNA* mutations in induced pluripotent stem cells (iPSCs) derived from HGPS patients, opening an avenue toward future cell therapies (Liu et al., 2011b). Further studies are necessary to validate the idea that reinforcement of the nuclear architecture can delay normal aging.

Overview

There is extensive evidence that genomic damage accompanies aging and that its artificial induction can provoke aspects of accelerated aging. In the case of the machinery that ensures faithful chromosomal segregation, there is genetic evidence that its enhancement can extend longevity in mammals (Baker et al., 2013). Also, in the particular case of progerias associated with nuclear architecture defects, there is proof of principle for treatments that can delay premature aging. Similar avenues should be explored to find interventions that reinforce other aspects of nuclear and mitochondrial genome stability, such as DNA repair, that could have a positive impact on normal aging (telomeres constitute a particular case and are discussed separately).

Telomere Attrition

Accumulation of DNA damage with age appears to affect the genome near to randomly, but there are some chromosomal regions, such as telomeres, that are particularly susceptible to age-related deterioration (Blackburn et al., 2006) (Figure 2A). Replicative DNA polymerases lack the capacity to replicate completely the terminal ends of linear DNA molecules, a function that is proprietary of a specialized DNA polymerase known as telomerase. However, most mammalian somatic cells do not express telomerase, and this leads to the progressive and cumulative loss of telomere-protective sequences from chromosome ends. Telomere exhaustion explains the limited proliferative capacity of some types of in-vitro-cultured cells, the so-called replicative senescence, or Hayflick limit (Hayflick and Moorhead, 1961; Olovnikov, 1996). Indeed, ectopic expression of telomerase is sufficient to confer immortality to otherwise mortal cells without causing oncogenic transformation (Bodnar et al., 1998). Importantly, telomere shortening is also observed during normal aging both in human and in mice (Blasco, 2007a).

Telomeres are bound by a characteristic multiprotein complex known as shelterin (Palm and de Lange, 2008). A main function of this complex is to prevent the access of DNA repair proteins to the telomeres. Otherwise, telomeres would be “repaired” as DNA breaks leading to chromosome fusions. Due to their restricted DNA repair, DNA damage at telomeres is notably persistent and highly efficient in inducing senescence and/or apoptosis (Fumagalli et al., 2012; Hewitt et al., 2012).

Telomerase deficiency in humans is associated with premature development of diseases, such as pulmonary fibrosis, dyskeratosis congenita, and aplastic anemia, which involve the loss of the regenerative capacity of different tissues (Armanios and Blackburn, 2012). Telomere uncapping and rampant chromosome fusions can also result from deficiencies in shelterin components (Palm and de Lange, 2008). Shelterin mutations have been found in some cases of aplastic anemia and dyskeratosis congenita (Savage et al., 2008; Walne et al., 2008; Zhong et al., 2011). Various loss-of-function models for shelterin components are characterized by rapid decline of the regenerative capacity of tissues and accelerated aging, a phenomenon that occurs even in the presence of telomeres with a normal length (Martínez and Blasco, 2010).

Genetically modified animal models have established causal links between telomere loss, cellular senescence, and organismal aging. Thus, mice with shortened or lengthened telomeres exhibit decreased or increased lifespans, respectively (Armanios et al., 2009; Blasco et al., 1997; Herrera et al., 1999; Rudolph et al., 1999; Tomás-Loba et al., 2008). Recent evidence also indicates that aging can be reverted by telomerase activation. In particular, the premature aging of telomerase-deficient mice can be reverted when telomerase is genetically reactivated in these aged mice (Jaskelioff et al., 2011). Moreover, normal physiological aging can be delayed without increasing the incidence of cancer in adult wild-type mice by systemic viral transduction of telomerase (Bernardes de Jesus et al., 2012). In humans, recent meta-analyses have supported the existence of a strong relation between short telomeres and mortality risk, particularly at younger ages (Boonekamp et al., 2013).

Overview

Normal aging is accompanied by telomere attrition in mammals. Moreover, pathological telomere dysfunction accelerates aging in mice and humans, whereas experimental stimulation of telomerase can delay aging in mice, thus fulfilling all of the criteria for a hallmark of aging.

Epigenetic Alterations

A variety of epigenetic alterations affects all cells and tissues throughout life (Talens et al., 2012) (Figure 2B). Epigenetic changes involve alterations in DNA methylation patterns, post-translational modification of histones, and chromatin remodeling. Increased histone H4K16 acetylation, H4K20 trimethylation, or H3K4 trimethylation, as well as decreased H3K9 methylation or H3K27 trimethylation, constitute age-associated epigenetic marks (Fraga and Esteller, 2007; Han and Brunet, 2012). The multiple enzymatic systems assuring the generation and maintenance of epigenetic patterns include DNA methyltransferases, histone acetylases, deacetylases, methylases, and demethylases, as well as protein complexes implicated in chromatin remodeling.

Histone Modifications

Histone methylation meets the criteria for a hallmark of aging in invertebrates. Deletion of components of histone methylation complexes (for H3K4 and for H3K27) extends longevity in nematodes and flies, respectively (Greer et al., 2010; Siebold et al., 2010). Moreover, inhibition of histone demethylases (for H3K27) in worms may extend lifespan by targeting components of key longevity routes such as the insulin/IGF-1 signaling pathway (Jin et al., 2011). It is not yet clear whether manipulations of histone-modifying enzymes can influence aging through purely epigenetic mechanisms, by impinging on DNA repair and genome stability, or through transcriptional alterations affecting metabolic or signaling pathways outside of the nucleus.

Members of the sirtuin family of NAD-dependent protein deacetylases and ADP ribosyltransferases have been studied extensively as potential anti-aging factors. Interest in this family of proteins in relation to aging stems from a series of studies in yeast, flies, and worms, which reported that the single sirtuin gene of these organisms, named *Sir2*, had a remarkable longevity activity (Guarente, 2011). Overexpression of *Sir2* was first shown to extend replicative lifespan in *Saccharomyces*

cerevisiae (Kaeberlein et al., 1999), and subsequent reports indicated that enhanced expression of the worm (*sir-2.1*) and fly (*dSir2*) orthologs could extend lifespan in both invertebrate model systems (Rogina and Helfand, 2004; Tissenbaum and Guarente, 2001). These findings have recently been called into question, however, with the report that the lifespan extension originally observed in the worm and fly studies was mostly due to confounding genetic background differences and not to the overexpression of *sir-2.1* or *dSir2*, respectively (Burnett et al., 2011). In fact, careful reassessments indicate that overexpression of *sir-2.1* only results in modest lifespan extension in *C. elegans* (Viswanathan and Guarente, 2011).

Regarding mammals, several of the seven mammalian sirtuin paralogs can ameliorate various aspects of aging in mice (Houtkooper et al., 2012; Sebastián et al., 2012). In particular, transgenic overexpression of mammalian SIRT1, which is the closest homolog to invertebrate Sir2, improves aspects of health during aging but does not increase longevity (Herranz et al., 2010). The mechanisms involved in the beneficial effects of SIRT1 are complex and interconnected, including improved genomic stability (Oberdoerffer et al., 2008; Wang et al., 2008) and enhanced metabolic efficiency (Nogueiras et al., 2012) (see “Deregulated Nutrient Sensing”). More compelling evidence for a sirtuin-mediated pro-longevity role in mammals has been obtained for SIRT6, which regulates genomic stability, NF- κ B signaling, and glucose homeostasis through histone H3K9 deacetylation (Kanfi et al., 2010; Kawahara et al., 2009; Zhong et al., 2010). Mutant mice that are deficient in SIRT6 exhibit accelerated aging (Mostoslavsky et al., 2006), whereas male transgenic mice overexpressing SIRT6 have a longer lifespan than control animals, associated with reduced serum IGF-1 and other indicators of IGF-1 signaling (Kanfi et al., 2012). Interestingly, the mitochondria-located sirtuin SIRT3 has been reported to mediate some of the beneficial effects of dietary restriction (DR) in longevity, though its effects are not due to histone modifications but, rather, due to the deacetylation of mitochondrial proteins (Someya et al., 2010). Very recently, overexpression of SIRT3 has been reported to improve the regenerative capacity of aged hematopoietic stem cells (Brown et al., 2013). Therefore, in mammals, at least three members of the sirtuin family—SIRT1, SIRT3 and SIRT6—contribute to healthy aging.

DNA Methylation

The relationship between DNA methylation and aging is complex. Early studies described an age-associated global hypomethylation, but subsequent analyses revealed that several loci, including those corresponding to various tumor suppressor genes and Polycomb target genes, actually become hypermethylated with age (Maegawa et al., 2010). Cells from patients and mice with progeroid syndromes exhibit DNA methylation patterns and histone modifications that largely recapitulate those found in normal aging (Osorio et al., 2010; Shumaker et al., 2006). All of these epigenetic defects or epimutations accumulated throughout life may specifically affect the behavior and functionality of stem cells (Pollina and Brunet, 2011) (see “Stem Cell Exhaustion”). Nevertheless, there is no direct experimental demonstration thus far that organismal lifespan can be extended by altering patterns of DNA methylation.

Chromatin Remodeling

DNA- and histone-modifying enzymes act in concert with key chromosomal proteins, such as the heterochromatin protein 1 α (HP1 α), and chromatin remodeling factors, such as Polycomb group proteins or the NuRD complex, whose levels are diminished in both normally and pathologically aged cells (Pegoraro et al., 2009; Pollina and Brunet, 2011). Along with the above discussed epigenetic modifications in histones and DNA methylation, alterations in these epigenetic factors determine changes in chromatin architecture, such as global heterochromatin loss and redistribution, which constitute characteristic features of aging (Oberdoerffer and Sinclair, 2007; Tsurumi and Li, 2012). The causal relevance of these chromatin alterations in aging is supported by the finding that flies with loss-of-function mutations in HP1 α have a shortened lifespan, whereas overexpression of this heterochromatin protein extends longevity in flies and delays the muscular deterioration characteristic of old age (Larson et al., 2012).

Supporting the functional relevance of epigenetically mediated chromatin alterations in aging, there is a notable connection between heterochromatin formation at repeated DNA domains and chromosomal stability. In particular, heterochromatin assembly at pericentric regions requires trimethylation of histones H3K9 and H4K20, as well as HP1 α binding, and is important for chromosomal stability (Schotta et al., 2004). Mammalian telomeric repeats are also enriched for these chromatin modifications, indicating that chromosome ends are assembled into heterochromatin domains (Blasco, 2007b; Gonzalez et al., 2006). Subtelomeric regions also show features of constitutive heterochromatin, including H3K9 and H4K20 trimethylation, HP1 α binding, and DNA hypermethylation. Thus, epigenetic alterations can directly impinge on the regulation of telomere length, one of the hallmarks of aging.

Transcriptional Alterations

Aging is associated with an increase in transcriptional noise (Bahar et al., 2006) and an aberrant production and maturation of many mRNAs (Harries et al., 2011; Nicholas et al., 2010). Microarray-based comparisons of young and old tissues from several species have identified age-related transcriptional changes in genes encoding key components of inflammatory, mitochondrial, and lysosomal degradation pathways (de Magalhães et al., 2009). These aging-associated transcriptional signatures also affect noncoding RNAs, including a class of miRNAs (gero-miRs) that is associated with the aging process and influences lifespan by targeting components of longevity networks or by regulating stem cell behavior (Boulias and Horvitz, 2012; Toledano et al., 2012; Ugalde et al., 2011). Gain- and loss-of-function studies have confirmed the capacity of several miRNAs to modulate longevity in *Drosophila melanogaster* and *C. elegans* (Liu et al., 2012; Shen et al., 2012; Smith-Vikos and Slack, 2012).

Reversion of Epigenetic Changes

Unlike DNA mutations, epigenetic alterations are—at least theoretically—reversible, hence offering opportunities for the design of novel anti-aging treatments (Freije and López-Otín, 2012; Rando and Chang, 2012). Restoration of physiological H4 acetylation through administration of histone deacetylase inhibitors avoids the manifestation of age-associated memory impairment

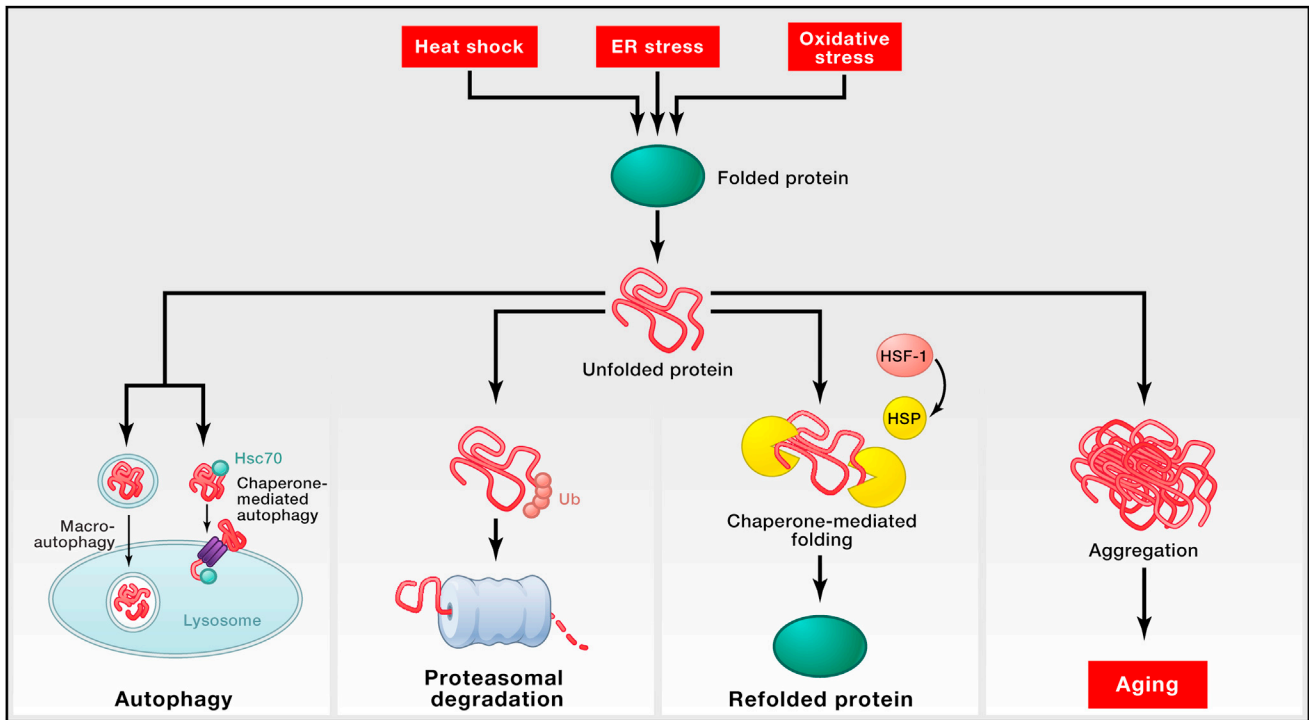


Figure 3. Loss of Proteostasis

Endogenous and exogenous stress causes the unfolding of proteins (or impairs proper folding during protein synthesis). Unfolded proteins are usually refolded by heat-shock proteins (HSP) or are targeted to destruction by the ubiquitin-proteasome or lysosomal (autophagic) pathways. The autophagic pathways include recognition of unfolded proteins by the chaperone Hsc70 and their subsequent import into lysosomes (chaperone-mediated autophagy) or sequestration of damaged proteins and organelles in autophagosomes that later fuse with lysosomes (macroautophagy). Failure to refold or degrade unfolded proteins can lead to their accumulation and aggregation, resulting in proteotoxic effects.

in mice (Peleg et al., 2010), indicating that reversion of epigenetic changes may have neuroprotective effects. Inhibitors of histone acetyltransferases also ameliorate the premature aging phenotypes of progeroid mice and extend their lifespan (Krishnan et al., 2011). Moreover, the recent discovery of transgenerational epigenetic inheritance of longevity in *C. elegans* suggests that manipulation of specific chromatin modifications in parents can induce an epigenetic memory of longevity in their descendants (Greer et al., 2011). Conceptually similar to histone acetyltransferase inhibitors, histone deacetylase activators may conceivably promote longevity. Resveratrol has been extensively studied in relation to aging, and among its multiple mechanisms of action are the upregulation of SIRT1 activity, as well as other effects associated with energetic deficits (see “Mitochondrial Dysfunction”).

Overview

There are multiple lines of evidence suggesting that aging is accompanied by epigenetic changes and that epigenetic perturbations can provoke progeroid syndromes in model organisms. Furthermore, SIRT6 exemplifies an epigenetically relevant enzyme whose loss of function reduces longevity and whose gain of function extends longevity in mice (Kanfi et al., 2012; Mostoslavsky et al., 2006). Collectively, these works suggest that understanding and manipulating the epigenome holds promise for improving age-related pathologies and extending healthy lifespan.

Loss of Proteostasis

Aging and some aging-related diseases are linked to impaired protein homeostasis or proteostasis (Powers et al., 2009) (Figure 3). All cells take advantage of an array of quality control mechanisms to preserve the stability and functionality of their proteomes. Proteostasis involves mechanisms for the stabilization of correctly folded proteins—most prominently, the heat-shock family of proteins—and mechanisms for the degradation of proteins by the proteasome or the lysosome (Hartl et al., 2011; Koga et al., 2011; Mizushima et al., 2008). Moreover, there are regulators of age-related proteotoxicity, such as MOAG-4, that act through an alternative pathway distinct from molecular chaperones and proteases (van Ham et al., 2010). All of these systems function in a coordinated fashion to restore the structure of misfolded polypeptides or to remove and degrade them completely, thus preventing the accumulation of damaged components and assuring the continuous renewal of intracellular proteins. Accordingly, many studies have demonstrated that proteostasis is altered with aging (Koga et al., 2011). Additionally, chronic expression of unfolded, misfolded, or aggregated proteins contributes to the development of some age-related pathologies, such as Alzheimer’s disease, Parkinson’s disease, and cataracts (Powers et al., 2009).

Chaperone-Mediated Protein Folding and Stability

The stress-induced synthesis of cytosolic and organelle-specific chaperones is significantly impaired in aging (Calderwood et al.,

2009). A number of animal models support a causative impact of chaperone decline on longevity. In particular, transgenic worms and flies overexpressing chaperones are long-lived (Morrow et al., 2004; Walker and Lithgow, 2003). Also, mutant mice deficient in a cochaperone of the heat-shock family exhibit accelerated aging phenotypes, whereas long-lived mouse strains show a marked upregulation of some heat-shock proteins (Min et al., 2008; Swindell et al., 2009). Moreover, activation of the master regulator of the heat-shock response, the transcription factor HSF-1, increases longevity and thermotolerance in nematodes (Chiang et al., 2012; Hsu et al., 2003), while amyloid-binding components can maintain proteostasis during aging and extend lifespan (Alavez et al., 2011). In mammalian cells, deacetylation of HSF-1 by SIRT1 potentiates the transactivation of heat-shock genes such as Hsp70, whereas downregulation of SIRT1 attenuates the heat-shock response (Westerheide et al., 2009).

Several approaches for maintaining or enhancing proteostasis aim at activating protein folding and stability mediated by chaperones. Pharmacological induction of the heat-shock protein Hsp72 preserves muscle function and delays progression of dystrophic pathology in mouse models of muscular dystrophy (Gehrig et al., 2012). Small molecules may be also employed as pharmacological chaperones to assure the refolding of damaged proteins and to improve age-related phenotypes in model organisms (Calamini et al., 2012).

Proteolytic Systems

The activities of the two principal proteolytic systems implicated in protein quality control—namely, the autophagy-lysosomal system and the ubiquitin-proteasome system—decline with aging (Rubinsztein et al., 2011; Tomaru et al., 2012), supporting the idea that collapsing proteostasis constitutes a common feature of old age.

Regarding autophagy, transgenic mice with an extra copy of the chaperone-mediated autophagy receptor LAMP2a do not experience aging-associated decline in autophagic activity and preserve improved hepatic function with aging (Zhang and Cuervo, 2008). Interventions using chemical inducers of macroautophagy (another type of autophagy different than chaperone-mediated autophagy) have spurred extraordinary interest after the discovery that constant or intermittent administration of the mTOR inhibitor rapamycin can increase the lifespan of middle-aged mice (Blagosklonny, 2011; Harrison et al., 2009). Notably, rapamycin delays multiple aspects of aging in mice (Wilkinson et al., 2012). The lifespan-extending effect of rapamycin is strictly dependent on the induction of autophagy in yeast, nematodes, and flies (Bjedov et al., 2010; Rubinsztein et al., 2011). However, similar evidence does not yet exist for the effects of rapamycin on mammalian aging, and other mechanisms, such as inhibition of the ribosomal S6 protein kinase 1 (S6K1) implicated in protein synthesis (Selman et al., 2009), could contribute to explain the longevity effects of rapamycin (see “Deregulated Nutrient Sensing”). Spermidine, another macroautophagy inducer that, in contrast to rapamycin, has no immunosuppressive side effects, also promotes longevity in yeast, flies, and worms via the induction of autophagy (Eisenberg et al., 2009). Similarly, nutrient supplementation with polyamine preparations containing spermidine or provision of a polyamine-producing gut flora increases longevity in mice (Matsumoto et al., 2011; Soda

et al., 2009). Dietary supplementation with ω -6 polyunsaturated fatty acids also extends lifespan in nematodes through autophagy activation (O’Rourke et al., 2013).

In relation to the proteasome, activation of EGF signaling extends longevity in nematodes by increasing the expression of various components of the ubiquitin-proteasome system (Liu et al., 2011a). Likewise, the enhancement of proteasome activity by deubiquitylase inhibitors or proteasome activators accelerates the clearance of toxic proteins in human cultured cells (Lee et al., 2010) and extends replicative lifespan in yeast (Kruegel et al., 2011). Moreover, increased expression of the proteasome subunit RPN-6 by the FOXO transcription factor DAF-16 confers proteotoxic stress resistance and extends lifespan in *C. elegans* (Vilchez et al., 2012).

Overview

There is evidence that aging is associated with perturbed proteostasis, and experimental perturbation of proteostasis can precipitate age-associated pathologies. There are also remarkable examples of genetic manipulations that improve proteostasis and delay aging in mammals (Zhang and Cuervo, 2008).

Deregulated Nutrient Sensing

The somatotrophic axis in mammals comprises the growth hormone (GH), which is produced by the anterior pituitary, and its secondary mediator, insulin-like growth factor 1 (IGF-1), produced in response to GH by many cell types, most notably hepatocytes. The intracellular signaling pathway of IGF-1 is the same as that elicited by insulin, which informs cells of the presence of glucose. For this reason, IGF-1 and insulin signaling are known as the “insulin and IGF-1 signaling” (IIS) pathway. Remarkably, the IIS pathway is the most conserved aging-controlling pathway in evolution, and among its multiple targets are the FOXO family of transcription factors and the mTOR complexes, which are also involved in aging and conserved through evolution (Barzilai et al., 2012; Fontana et al., 2010; Kenyon, 2010). Genetic polymorphisms or mutations that reduce the functions of GH, IGF-1 receptor, insulin receptor, or downstream intracellular effectors such as AKT, mTOR, and FOXO have been linked to longevity, both in humans and in model organisms, further illustrating the major impact of trophic and bioenergetic pathways on longevity (Barzilai et al., 2012; Fontana et al., 2010; Kenyon, 2010) (Figure 4A).

Consistent with the relevance of deregulated nutrient sensing as a hallmark of aging, dietary restriction (DR) increases lifespan or healthspan in all investigated eukaryote species, including nonhuman primates (Colman et al., 2009; Fontana et al., 2010; Mattison et al., 2012).

The Insulin- and IGF-1-Signaling Pathway

Multiple genetic manipulations that attenuate signaling intensity at different levels of the IIS pathway consistently extend the lifespan of worms, flies, and mice (Fontana et al., 2010). Genetic analyses indicate that this pathway mediates part of the beneficial effects of DR on longevity in worms and flies (Fontana et al., 2010). Among the downstream effectors of the IIS pathway, the most relevant one for longevity in worms and flies is the transcription factor FOXO (Kenyon et al., 1993; Slack et al., 2011). In mice, there are four FOXO members, but the effect of their overexpression on longevity and their role in mediating increased healthspan through reduced IIS have not yet been determined.

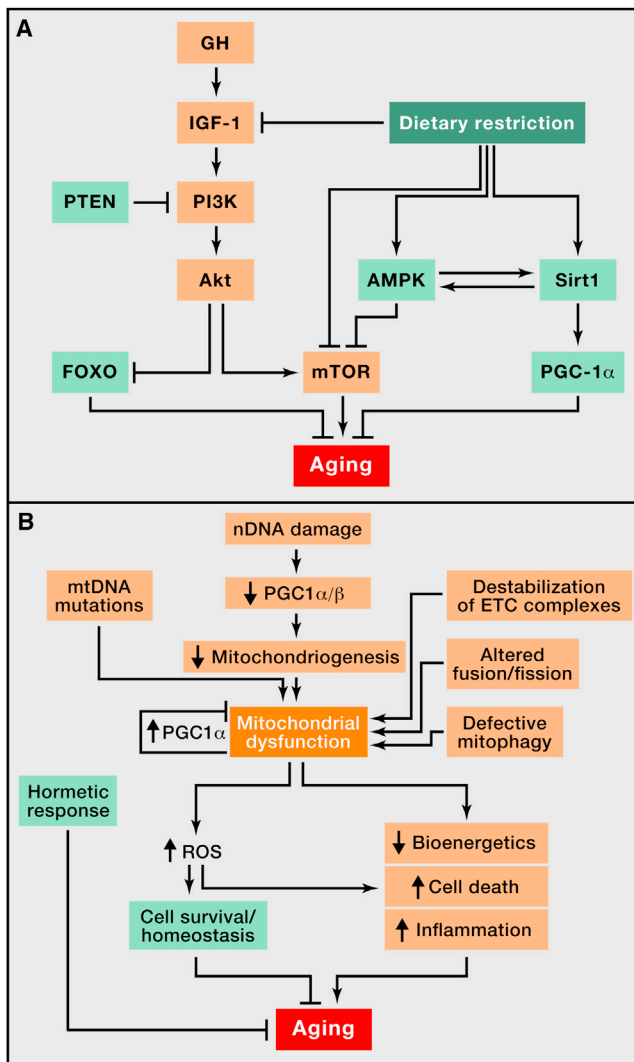


Figure 4. Metabolic Alterations

(A) Deregulated nutrient sensing. Overview of the somatroph axis involving growth hormone (GH) and the insulin/insulin growth factor 1 (IGF-1) signaling pathway and its relationship to dietary restriction and aging. Molecules that favor aging are shown in orange, and molecules with anti-aging properties are shown in light green.

(B) Mitochondrial dysfunction. Mitochondrial function becomes perturbed by aging-associated mtDNA mutations, reduced mitochondriogenesis, destabilization of the electron transport chain (ETC) complexes, altered mitochondrial dynamics, or defective quality control by mitophagy. Stress signals and defective mitochondrial function generate ROS that, below a certain threshold, induce survival signals to restore cellular homeostasis but, at higher or continued levels, can contribute to aging. Similarly, mild mitochondrial damage can induce a hormetic response (mitohormesis) that triggers adaptive compensatory processes.

Mouse FOXO1 is required for the tumor suppressive effect of DR (Yamaza et al., 2010), but it is not yet known whether this factor is involved in DR-mediated lifespan extension. More recently, mice with increased dosage of the tumor suppressor *PTEN* have been reported to exhibit a general downmodulation of the IIS pathway and an increased energy expenditure that is associated with improved mitochondrial oxidative metabolism, as well as with

an enhanced activity of the brown adipose tissue (Garcia-Cao et al., 2012; Ortega-Molina et al., 2012). In line with other mouse models with decreased IIS activity, *Pten*-overexpressing mice, as well as hypomorphic PI3K mice, show an increased longevity (Foukas et al., 2013; Ortega-Molina et al., 2012).

Paradoxically, GH and IGF-1 levels decline during normal aging, as well as in mouse models of premature aging (Schumacher et al., 2008). Thus, a decreased IIS is a common characteristic of both physiological and accelerated aging, whereas a constitutively decreased IIS extends longevity. These apparently contradictory observations can be accommodated under a unifying model by which IIS downmodulation reflects a defensive response aimed at minimizing cell growth and metabolism in the context of systemic damage (Garinis et al., 2008). According to this view, organisms with a constitutively decreased IIS can survive longer because they have lower rates of cell growth and metabolism and, hence, lower rates of cellular damage. Along the same lines, physiologically or pathologically aged organisms decrease IIS in an attempt to extend their lifespan. However, and this is a concept that will recur in the following sections, defensive responses against aging may have the risk of eventually becoming deleterious and aggravating aging. Thus, extremely low levels of IIS signaling are incompatible with life, as exemplified by mouse null mutations in the PI3K or AKT kinases that are embryonic lethal (Renner and Carnero, 2009). Also, there are cases of progeroid mice with very low levels of IGF-1, in which supplementation of IGF-1 can ameliorate premature aging (Mariño et al., 2010).

Other Nutrient-Sensing Systems: mTOR, AMPK, and Sirtuins

In addition to the IIS pathway that participates in glucose sensing, three additional related and interconnected nutrient-sensing systems are the focus of intense investigation: mTOR, for the sensing of high amino acid concentrations; AMPK, which senses low-energy states by detecting high AMP levels; and sirtuins, which sense low-energy states by detecting high NAD⁺ levels (Houtkooper et al., 2010) (Figure 4A).

The mTOR kinase is part of two multiprotein complexes, mTORC1 and mTORC2, that regulate essentially all aspects of anabolic metabolism (Laplante and Sabatini, 2012). Genetic downregulation of mTORC1 activity in yeast, worms, and flies extends longevity and attenuates further longevity benefits from DR, suggesting that mTOR inhibition phenocopies DR (Johnson et al., 2013). In mice, treatment with rapamycin also extends longevity in what is considered to be the most robust chemical intervention to increase lifespan in mammals (Harrison et al., 2009). Genetically modified mice with low levels of mTORC1 activity but normal levels of mTORC2 have increased lifespan (Lamming et al., 2012), and mice deficient in S6K1 (a main mTORC1 substrate) are also long-lived (Selman et al., 2009). Therefore, the downregulation of mTORC1/S6K1 appears as the critical mediator of mammalian longevity in relation to mTOR. Moreover, mTOR activity increases during aging in mouse hypothalamic neurons, contributing to age-related obesity, which is reversed by direct infusion of rapamycin to the hypothalamus (Yang et al., 2012). These observations, together with those involving the IIS pathway, indicate that intense trophic and anabolic activity, signaled through the IIS

or the mTORC1 pathways, are major accelerators of aging. Although inhibition of TOR activity clearly has beneficial effects during aging, it also has undesirable side effects, such as impaired wound healing, insulin resistance, cataracts, and testicular degeneration in mice (Wilkinson et al., 2012). It will thus be important to understand the mechanisms involved in order to determine the extent to which beneficial and damaging effects of TOR inhibition can be separated from each other.

The other two nutrient sensors, AMPK and sirtuins, act in the opposite direction to IIS and mTOR, meaning that they signal nutrient scarcity and catabolism instead of nutrient abundance and anabolism. Accordingly, their upregulation favors healthy aging. AMPK activation has multiple effects on metabolism and, remarkably, shuts off mTORC1 (Alers et al., 2012). There is evidence indicating that AMPK activation may mediate lifespan extension following metformin administration to worms and mice (Anisimov et al., 2011; Mair et al., 2011; Onken and Driscoll, 2010). The role of sirtuins in lifespan regulation has been discussed above (see “Epigenetic Alterations”). In addition, SIRT1 can deacetylate and activate the PPAR γ coactivator 1 α (PGC-1 α) (Rodgers et al., 2005). PGC-1 α orchestrates a complex metabolic response that includes mitochondrial biogenesis, enhanced antioxidant defenses, and improved fatty acid oxidation (Fernandez-Marcos and Auwerx, 2011). Moreover, SIRT1 and AMPK can engage in a positive feedback loop, thus connecting both sensors of low-energy states into a unified response (Price et al., 2012).

Overview

Collectively, current available evidence strongly supports the idea that anabolic signaling accelerates aging and decreased nutrient signaling extends longevity (Fontana et al., 2010). Further, a pharmacological manipulation that mimics a state of limited nutrient availability, such as rapamycin, can extend longevity in mice (Harrison et al., 2009).

Mitochondrial Dysfunction

As cells and organisms age, the efficacy of the respiratory chain tends to diminish, thus increasing electron leakage and reducing ATP generation (Green et al., 2011) (Figure 4B). The relation between mitochondrial dysfunction and aging has been long suspected, but dissecting its details remains as a major challenge for aging research.

Reactive Oxygen Species

The mitochondrial free radical theory of aging proposes that the progressive mitochondrial dysfunction that occurs with aging results in increased production of ROS, which in turn causes further mitochondrial deterioration and global cellular damage (Harman, 1965). Multiple data support a role for ROS in aging, but we focus here on the developments of the last 5 years, which have forced an intense re-evaluation of the mitochondrial free radical theory of aging (Hekimi et al., 2011). Of particular impact has been the unexpected observation that increased ROS may prolong lifespan in yeast and *C. elegans* (Doonan et al., 2008; Mesquita et al., 2010; Van Raamsdonk and Hekimi, 2009). Similarly unexpected, genetic manipulations in mice that increase mitochondrial ROS and oxidative damage do not accelerate aging (Van Remmen et al., 2003; Zhang et al., 2009), mice with increased antioxidant defenses do not present an extended life-

span (Pérez et al., 2009), and, finally, genetic manipulations that impair mitochondrial function but do not increase ROS accelerate aging (Edgar et al., 2009; Hiona et al., 2010; Kujoth et al., 2005; Trifunovic et al., 2004; Vermulst et al., 2008). These and other data have paved the way to a reconsideration of the role of ROS in aging (Ristow and Schmeisser, 2011). Indeed, parallel and separate to the work on the damaging effects of ROS, the field of intracellular signaling has accumulated solid evidence for the role of ROS in triggering proliferation and survival in response to physiological signals and stress conditions (Sena and Chandel, 2012). The two lines of evidence can be harmonized if ROS is regarded as a stress-elicited survival signal conceptually similar to AMP or NAD⁺ (see “Deregulated Nutrient Sensing”). In this sense, the primary effect of ROS will be the activation of compensatory homeostatic responses. As chronological age advances, cellular stress and damage increase and the levels of ROS increase in parallel in an attempt to maintain survival. Beyond a certain threshold, ROS levels betray their original homeostatic purpose and eventually aggravate, rather than alleviate, the age-associated damage (Hekimi et al., 2011). This new conceptual framework may accommodate apparently conflicting evidence regarding the positive, negative, or neutral effects of ROS on aging.

Mitochondrial Integrity and Biogenesis

Dysfunctional mitochondria can contribute to aging independently of ROS, as exemplified by studies with mice that are deficient in DNA polymerase γ (Edgar et al., 2009; Hiona et al., 2010) (see “Genomic Instability”). This could happen through a number of mechanisms; for example, mitochondrial deficiencies may affect apoptotic signaling by increasing the propensity of mitochondria to permeabilize in response to stress (Kroemer et al., 2007) and trigger inflammatory reactions by favoring ROS-mediated and/or permeabilization-facilitated activation of inflammasomes (Green et al., 2011). Also, mitochondrial dysfunction may directly impact cellular signaling and interorganellar cross-talk by affecting the interface between the outer mitochondrial membrane and the endoplasmic reticulum (Raffaello and Rizzuto, 2011).

The reduced efficiency of mitochondrial bioenergetics with aging may result from multiple converging mechanisms, including reduced biogenesis of mitochondria—for instance, as a consequence of telomere attrition in telomerase-deficient mice, with subsequent p53-mediated repression of PGC-1 α and PGC-1 β (Sahin and DePinho, 2012). This mitochondrial decline also occurs during physiological aging in wild-type mice and can be partially reversed by telomerase activation (Bernardes de Jesus et al., 2012). SIRT1 modulates mitochondrial biogenesis through a process involving the transcriptional coactivator PGC-1 α (Rodgers et al., 2005) and the removal of damaged mitochondria by autophagy (Lee et al., 2008). SIRT3, which is the main mitochondrial deacetylase (Lombard et al., 2007), targets many enzymes involved in energy metabolism, including components of the respiratory chain, tricarboxylic acid cycle, ketogenesis, and fatty acid β -oxidation (Giralt and Villarroya, 2012). SIRT3 may also directly control the rate of ROS production by deacetylating manganese superoxide dismutase, a major mitochondrial antioxidant enzyme (Qiu et al., 2010; Tao et al., 2010). Collectively, these results support the idea that telomeres and sirtuins

may control mitochondrial function and thus play a protective role against age-associated diseases.

Other mechanisms causing defective bioenergetics include accumulation of mutations and deletions in mtDNA, oxidation of mitochondrial proteins, destabilization of the macromolecular organization of respiratory chain (super)complexes, changes in the lipid composition of mitochondrial membranes, alterations in mitochondrial dynamics resulting from imbalance of fission and fusion events, and defective quality control by mitophagy, an organelle-specific form of macroautophagy that targets deficient mitochondria for proteolytic degradation (Wang and Klionsky, 2011). The combination of increased damage and reduced turnover in mitochondria, due to lower biogenesis and reduced clearance, may contribute to the aging process (Figure 4B).

Interestingly, endurance training and alternate-day fasting may improve healthspan through their capacity to avoid mitochondrial degeneration (Castello et al., 2011; Safdar et al., 2011). It is tempting to speculate that these beneficial effects are mediated, at least in part, through the induction of autophagy, for which both endurance training and fasting constitute potent triggers (Rubinsztein et al., 2011). However, autophagy induction is probably not the sole mechanism through which a healthy lifestyle can retard aging, as depending on the precise DR regime, additional longevity pathways can be activated (Kenny, 2010).

Mitohormesis

Mitochondrial dysfunctions during aging are also connected with hormesis, a concept on which a number of aging research lines have recently converged (Calabrese et al., 2011). According to this concept, mild toxic treatments trigger beneficial compensatory responses that surpass the repair of the triggering damage and actually produce an improvement in cellular fitness when compared to the starting predamage conditions. Thus, although severe mitochondrial dysfunction is pathogenic, mild respiratory deficiencies may increase lifespan, perhaps due to a hormetic response (Haigis and Yankner, 2010). Hormetic reactions may trigger a mitochondrial defensive response both in the same tissue in which mitochondria are defective and even in distant tissues, as shown in *C. elegans* (Durieux et al., 2011). There is compelling evidence that compounds such as metformin and resveratrol are mild mitochondrial poisons that induce a low-energy state characterized by increased AMP levels and activation of AMPK (Hawley et al., 2010). Importantly, metformin extends lifespan in *C. elegans* through the induction of a compensatory stress response mediated by AMPK and the master antioxidant regulator NRF2 (Onken and Driscoll, 2010). Recent studies have also shown that metformin retards aging in worms by impairing folate and methionine metabolism of their intestinal microbiome (Cabreiro et al., 2013). Regarding mammals, metformin can increase mouse lifespan when administered from early life (Anisimov et al., 2011). In the cases of resveratrol and the sirtuin activator SRT1720, there is convincing evidence that they protect from metabolic damage and improve mitochondrial respiration in a PGC-1 α -dependent fashion (Baur et al., 2006; Feige et al., 2008; Lagouge et al., 2006; Minor et al., 2011), although resveratrol does not extend mouse lifespan under normal dietary conditions (Pearson et al., 2008; Strong et al., 2013). Further support for the role of PGC-1 α in longevity comes

from the observation that PGC-1 α overexpression suffices to extend *Drosophila* lifespan in association with improved mitochondrial activity (Rera et al., 2011). Finally, mitochondrial uncoupling—either genetically through the overexpression of the uncoupling protein UCP1 or by administration of the chemical uncoupler 2-4-dinitrophenol—can increase lifespan in flies and mice (Caldeira da Silva et al., 2008; Fridell et al., 2009; Gates et al., 2007; Mookerjee et al., 2010).

Overview

Mitochondrial function has a profound impact on the aging process. Mitochondrial dysfunction can accelerate aging in mammals (Kujoth et al., 2005; Trifunovic et al., 2004; Vermulst et al., 2008), but it is less clear whether improving mitochondrial function, for example through mitohormesis, can extend lifespan in mammals, though suggestive evidence in this sense already exists.

Cellular Senescence

Cellular senescence can be defined as a stable arrest of the cell cycle coupled to stereotyped phenotypic changes (Campisi and d'Adda di Fagagna, 2007; Collado et al., 2007; Kuilman et al., 2010) (Figure 5A). This phenomenon was originally described by Hayflick in human fibroblasts serially passaged in culture (Hayflick and Moorhead, 1961). Today, we know that the senescence observed by Hayflick is caused by telomere shortening (Bodnar et al., 1998), but there are other aging-associated stimuli that trigger senescence independently of this telomeric process. Most notably, nontelomeric DNA damage and derepression of the *INK4/ARF* locus, both of which progressively occur with chronological aging, are also capable of inducing senescence (Collado et al., 2007). The accumulation of senescent cells in aged tissues has been often inferred using surrogate markers such as DNA damage. Some studies have directly used senescence-associated β -galactosidase (SABG) to identify senescence in tissues (Dimri et al., 1995). Of note, a detailed and parallel quantification of SABG and DNA damage in liver produced comparable quantitative data, yielding a total of ~8% senescent cells in young mice and ~17% in very old mice (Wang et al., 2009). Similar results were obtained in the skin, lung, and spleen, but no changes were observed in heart, skeletal muscle, and kidney (Wang et al., 2009). Based on these data, it is clear that cellular senescence is not a generalized property of all tissues in aged organisms. In the case of senescent tumor cells, there is good evidence that they are subjected to strict immune surveillance and are efficiently removed by phagocytosis (Hoenicke and Zender, 2012; Kang et al., 2011; Xue et al., 2007). Conceivably, the accumulation of senescent cells with aging can reflect an increase in the rate of generation of senescent cells and/or a decrease in their rate of clearance—for example, as a consequence of an attenuated immune response.

Because the number of senescent cells increases with aging, it has been widely assumed that senescence contributes to aging. However, this view undervalues what conceivably is the primary purpose of senescence, which is to prevent the propagation of damaged cells and to trigger their demise by the immune system. Therefore, it is possible that senescence is a beneficial compensatory response that contributes to rid tissues from damaged and potentially oncogenic cells. This cellular

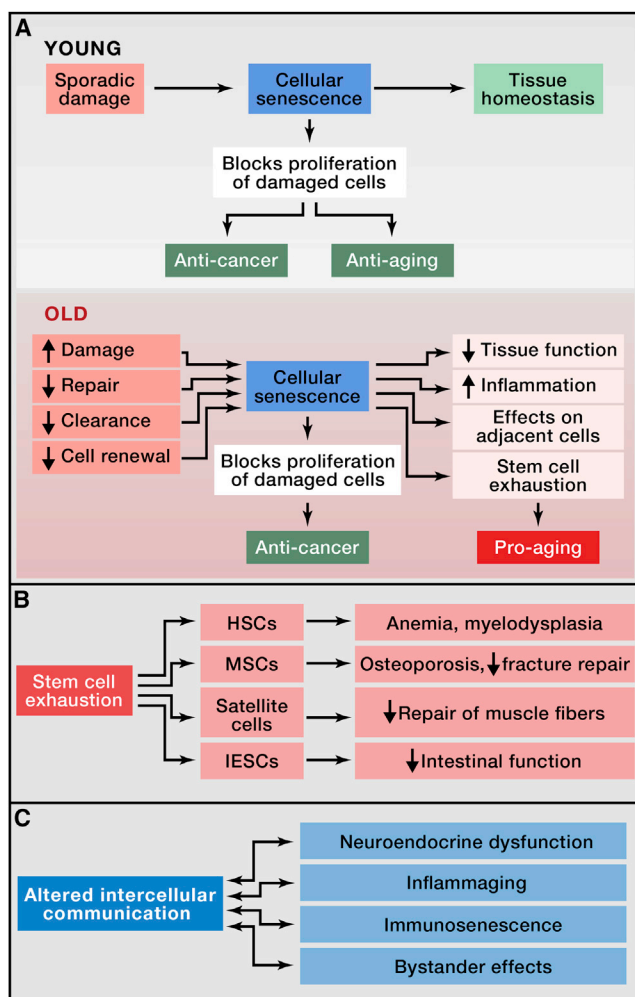


Figure 5. Cellular Senescence, Stem Cell Exhaustion, and Altered Intercellular Communication

(A) Cellular senescence. In young organisms, cellular senescence prevents the proliferation of damaged cells, thus protecting from cancer and contributing to tissue homeostasis. In old organisms, the pervasive damage and the deficient clearance of senescent cells result in their accumulation, and this has a number of deleterious effects on tissue homeostasis that contribute to aging. (B) Stem cell exhaustion. Consequences of the exhaustion of hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), satellite cells, and intestinal epithelial stem cells (IESCs) are exemplified. (C) Altered intercellular communication. Examples of altered intercellular communication associated with aging.

checkpoint, however, requires an efficient cell replacement system that involves clearance of senescent cells and mobilization of progenitors to re-establish cell numbers. In aged organisms, this turnover system may become inefficient or may exhaust the regenerative capacity of progenitor cells, eventually resulting in the accumulation of senescent cells that may aggravate the damage and contribute to aging (Figure 5A).

In recent years, it has been appreciated that senescent cells manifest dramatic alterations in their secretome, which is particularly enriched in proinflammatory cytokines and matrix metalloproteinases and is referred to as the “senescence-associated

secretory phenotype” (Kuilman et al., 2010; Rodier and Campisi, 2011). This proinflammatory secretome may contribute to aging (see “Intercellular Communication”).

The *INK4a/ARF* Locus and p53

In addition to DNA damage, excessive mitogenic signaling is the other stress most robustly associated to senescence. A recent account listed more than 50 oncogenic or mitogenic alterations that are able to induce senescence (Gorgoulis and Halazonetis, 2010). The number of mechanisms that implement senescence in response to this variety of oncogenic insults has also grown, but the originally reported p16^{INK4a}/Rb and p19^{ARF}/p53 pathways remain, in general, the most important ones (Serrano et al., 1997). The relevance of these pathways for aging becomes even more striking when considering that the levels of p16^{INK4a} (and, to a lower extent, also p19^{ARF}) correlate with the chronological age of essentially all tissues analyzed both in mice and humans (Krishnamurthy et al., 2004; Ressler et al., 2006). We are not aware of any other gene or protein whose expression is so robustly correlated with chronological aging across tissues, across species, and with a range of variation that, on average, is one order of magnitude between young and old tissues. Both p16^{INK4a} and p19^{ARF} are encoded by the same genetic locus, the *INK4a/ARF* locus. A recent meta-analysis of more than 300 genome-wide association studies (GWAS) identified the *INK4a/ARF* locus as the genomic locus that is genetically linked to the highest number of age-associated pathologies, including several types of cardiovascular diseases, diabetes, glaucoma, and Alzheimer’s disease (Jeck et al., 2012). These observations place the *INK4a/ARF* locus as the best documented gene that controls human aging and aging-associated pathologies. A critical pending issue is to determine whether the disease-associated *INK4a/ARF* alleles present gain or loss of function.

The critical role of p16^{INK4a} and p53 in the induction of cell senescence has favored the hypothesis that p16^{INK4a}-induced and p53-induced senescence contribute to physiological aging. According to this view, the pro-aging activity of p16^{INK4a} and p53 would be a tolerable toll compared to their benefits in tumor suppression. In support of this, mutant mice with premature aging due to extensive and persistent damage present dramatic levels of senescence, and their progeroid phenotypes are ameliorated by elimination of p16^{INK4a} or p53. This is the case with mice deficient in BRCA1 (Cao et al., 2003), with a mouse model of HGPS (Varela et al., 2005), and with mice with defective chromosomal stability due to a hypomorphic mutation of BubR1 (Baker et al., 2011). However, other evidences suggest a more complicated picture. In contrast to their anticipated pro-aging role, mice with a mild and systemic increase in p16^{INK4a}, p19^{ARF}, or p53 tumor suppressors exhibit extended longevity, which cannot be accounted for by their lower cancer incidence (Matheu et al., 2007, 2009). Also, elimination of p53 aggravates the phenotypes of some progeroid mutant mice (Begus-Nahrmann et al., 2009; Murga et al., 2009; Ruzankina et al., 2009). Again, as discussed above for senescence, the activation of p53 and *INK4a/ARF* can be regarded as a beneficial compensatory response aimed at avoiding the propagation of damaged cells and its consequences on aging and cancer. However, when damage is pervasive, the regenerative capacity of tissues can be exhausted or saturated, and under these extreme conditions, the p53 and

INK4a/ARF responses can become deleterious and accelerate aging.

Overview

We propose that cellular senescence is a beneficial compensatory response to damage that becomes deleterious and accelerates aging when tissues exhaust their regenerative capacity. Given these complexities, it is not possible to give a simple answer to the question of whether cell senescence fulfills the third ideal criteria for the definition of a hallmark. A moderate enhancement of the senescence-inducing tumor suppressor pathways may extend longevity (Matheu et al., 2007, 2009), and at the same time, elimination of senescent cells in an experimental progeria model delays age-related pathologies (Baker et al., 2011). Therefore, two interventions that are conceptually opposite are able to extend healthspan.

Stem Cell Exhaustion

The decline in the regenerative potential of tissues is one of the most obvious characteristics of aging (Figure 5B). For example, hematopoiesis declines with age, resulting in a diminished production of adaptive immune cells—a process termed immunosenescence—and in an increased incidence of anemia and myeloid malignancies (Shaw et al., 2010). A similar functional attrition of stem cells has been found in essentially all adult stem cell compartments, including the mouse forebrain (Molofsky et al., 2006), the bone (Gruber et al., 2006), or the muscle fibers (Conboy and Rando, 2012). Studies on aged mice have revealed an overall decrease in cell-cycle activity of hematopoietic stem cells (HSCs), with old HSCs undergoing fewer cell divisions than young HSCs (Rossi et al., 2007). This correlates with the accumulation of DNA damage (Rossi et al., 2007) and with the overexpression of cell-cycle inhibitory proteins such as p16^{INK4a} (Janzen et al., 2006). In fact, old *INK4a*^{-/-} HSCs exhibit better engraftment capacity and increased cell-cycle activity compared with old wild-type HSCs (Janzen et al., 2006). Telomere shortening is also an important cause of stem cell decline with aging in multiple tissues (Flores et al., 2005; Sharpless and DePinho, 2007). These are just examples of a much larger picture in which stem cell decline emerges as the integrative consequence of multiple types of damage.

Although deficient proliferation of stem and progenitor cells is obviously detrimental for the long-term maintenance of the organism, an excessive proliferation of stem and progenitor cells can also be deleterious by accelerating the exhaustion of stem cell niches. The importance of stem cell quiescence for the long-term functionality of stem cells has been compellingly demonstrated in the case of *Drosophila* intestinal stem cells, in which excessive proliferation leads to exhaustion and premature aging (Rera et al., 2011). A similar situation is encountered in *p21* null mice, which present premature exhaustion of HSCs and neural stem cells (Cheng et al., 2000; Kippin et al., 2005). In this regard, the induction of *INK4a* during aging (see “Cellular Senescence”) and the decrease of serum IGF-1 (see “Deregulated Nutrient Sensing”) may both reflect an attempt of the organism to preserve the quiescence of stem cells. Also, recent studies have shown that an increase in FGF2 signaling in the aged muscle stem cell niche results in the loss of quiescence and eventually in stem cell depletion and diminished regenera-

tive capacity, whereas suppression of this signaling pathway rescues these defects (Chakkalakal et al., 2012). This opens the possibility of designing strategies aimed at inhibiting FGF2 signaling to reduce stem cell exhaustion during aging. Along these lines, DR has been reported to increase intestinal and muscle stem functions (Cerletti et al., 2012; Yilmaz et al., 2012).

An important debate regarding the decline in stem cell function is the relative role of cell-intrinsic pathways compared to cell-extrinsic ones (Conboy and Rando, 2012). Recent work has provided strong support for the latter. In particular, transplantation of muscle-derived stem cells from young mice to progeroid mice extends lifespan and improves degenerative changes of these animals even in tissues in which donor cells are not detected, suggesting that their therapeutic benefit may derive from systemic effects caused by secreted factors (Lavasani et al., 2012). Furthermore, parabiosis experiments have demonstrated that the decline in neural and muscle stem cell function in old mice can be reversed by systemic factors from young mice (Conboy et al., 2005; Villeda et al., 2011).

Pharmacological interventions are also being explored to improve stem cell function. In particular, mTORC1 inhibition with rapamycin, which can postpone aging by improving proteostasis (see “Loss of Proteostasis”) and by affecting energy sensing (see “Deregulated Nutrient Sensing”), may also improve stem cell function in the epidermis, in the hematopoietic system, and in the intestine (Castilho et al., 2009; Chen et al., 2009; Yilmaz et al., 2012). This also illustrates the difficulty of disentangling the mechanistic basis for the anti-aging activity of rapamycin and underscores the interconnectedness between the different hallmarks of aging discussed here. It is also worth mentioning that it is possible to rejuvenate human senescent cells by pharmacological inhibition of the GTPase CDC42, whose activity is increased in aged HSCs (Florian et al., 2012).

Overview

Stem cell exhaustion unfolds as the integrative consequence of multiple types of aging-associated damages and likely constitutes one of the ultimate culprits of tissue and organismal aging. Recent promising studies suggest that stem cell rejuvenation may reverse the aging phenotype at the organismal level (Rando and Chang, 2012).

Altered Intercellular Communication

Beyond cell-autonomous alterations, aging also involves changes at the level of intercellular communication, be it endocrine, neuroendocrine, or neuronal (Laplante and Sabatini, 2012; Rando and Chang, 2012; Russell and Kahn, 2007; Zhang et al., 2013) (Figure 5C). Thus, neurohormonal signaling (e.g., renin-angiotensin, adrenergic, insulin-IGF1 signaling) tends to be deregulated in aging as inflammatory reactions increase, immunosurveillance against pathogens and premalignant cells declines, and the composition of the peri- and extracellular environment changes.

Inflammation

A prominent aging-associated alteration in intercellular communication is “inflammaging,” i.e., a smoldering proinflammatory phenotype that accompanies aging in mammals (Salminen et al., 2012). Inflammaging may result from multiple causes, such as the accumulation of proinflammatory tissue damage,

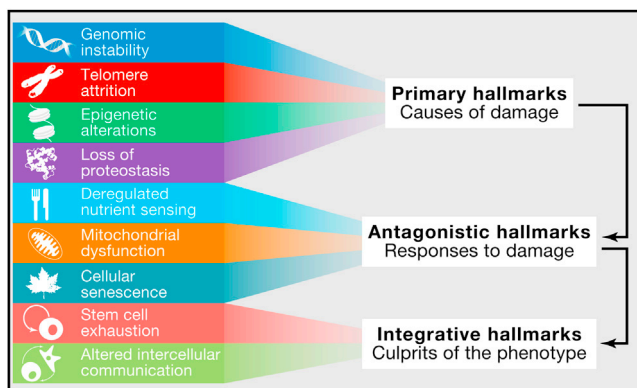


Figure 6. Functional Interconnections between the Hallmarks of Aging

The proposed nine hallmarks of aging are grouped into three categories. (Top) Those hallmarks considered to be the primary causes of cellular damage. (Middle) Those considered to be part of compensatory or antagonistic responses to the damage. These responses initially mitigate the damage, but eventually, if chronic or exacerbated, they become deleterious themselves. (Bottom) Integrative hallmarks that are the end result of the previous two groups of hallmarks and are ultimately responsible for the functional decline associated with aging.

the failure of an ever more dysfunctional immune system to effectively clear pathogens and dysfunctional host cells, the propensity of senescent cells to secrete proinflammatory cytokines (see “Cellular Senescence”), the enhanced activation of the NF- κ B transcription factor, or the occurrence of a defective autophagy response (Salminen et al., 2012). These alterations result in an enhanced activation of the NLRP3 inflammasome and other proinflammatory pathways, finally leading to increased production of IL-1 β , tumor necrosis factor, and interferons (Green et al., 2011; Salminen et al., 2012). Inflammation is also involved in the pathogenesis of obesity and type 2 diabetes, two conditions that contribute to and correlate with aging in the human population (Barzilai et al., 2012). Likewise, defective inflammatory responses play a critical role in atherosclerosis (Tabas, 2010). The recent finding that age-associated inflammation inhibits epidermal stem cell function (Doles et al., 2012) further supports the intricate concatenation of different hallmarks that reinforces the aging process. Paralleling inflammaging, the function of the adaptive immune system declines (Deeks, 2011). This immunosenescence may aggravate the aging phenotype at the systemic level due to the failure of the immune system to clear infectious agents, infected cells, and cells on the verge of malignant transformation. Moreover, one of the functions of the immune system is to recognize and eliminate senescent cells (see “Stem Cell Exhaustion”), as well as hyperploid cells that accumulate in aging tissues and premalignant lesions (Davoli and de Lange, 2011; Senovilla et al., 2012).

Global studies on the transcriptional landscape of aged tissues have also emphasized the relevance of inflammatory pathways in aging (de Magalhães et al., 2009; Lee et al., 2012). Overactivation of the NF- κ B pathway is one of these transcriptional signatures of aging, and conditional expression of an NF- κ B inhibitor in the aged skin of transgenic mice causes the phenotypic rejuvenation of this tissue, as well as the restoration

of the transcriptional signature corresponding to young age (Adler et al., 2007). Likewise, genetic and pharmacological inhibition of NF- κ B signaling prevents age-associated features in different mouse models of accelerated aging (Osorio et al., 2012; Tilstra et al., 2012). A novel link between inflammation and aging derives from the recent finding that inflammatory and stress responses activate NF- κ B in the hypothalamus and induce a signaling pathway that results in reduced production of gonadotropin-releasing hormone (GnRH) by neurons (Zhang et al., 2013). This GnRH decline can contribute to numerous aging-related changes such as bone fragility, muscle weakness, skin atrophy, and reduced neurogenesis. Consistently, GnRH treatment prevents aging-impaired neurogenesis and decelerates aging development in mice (Zhang et al., 2013). These findings suggest that the hypothalamus may modulate systemic aging by integrating NF- κ B-driven inflammatory responses with GnRH-mediated neuroendocrine effects.

Further *in vivo* evidence linking inflammation and aging derives from work on the mRNA decay factor AUF1, which is implicated in the cessation of the inflammatory response by mediating cytokine mRNA degradation (Pont et al., 2012). *AUF1*-deficient mice exhibit a marked cellular senescence and premature aging phenotype that can be rescued by re-expression of this RNA-binding factor. Interestingly, in addition to directing inflammatory cytokine mRNA decay, AUF1 contributes to maintaining telomere length by activating the expression of the telomerase catalytic subunit TERT (Pont et al., 2012), again demonstrating that one single factor may have a strong impact on different aging hallmarks.

A similar situation occurs with sirtuins, which may also have an impact on inflammatory responses associated with aging. Several studies have revealed that, by deacetylating histones and components of inflammatory signaling pathways such as NF- κ B, SIRT1 can downregulate inflammation-related genes (Xie et al., 2013). Consistent with these findings, reduction of SIRT1 levels correlates with the development and progression of many inflammatory diseases, and pharmacologic activation of SIRT1 may prevent inflammatory responses in mice (Gillum et al., 2011; Yao et al., 2012; Zhang et al., 2010). SIRT2 and SIRT6 may also downregulate the inflammatory response through deacetylation of NF- κ B subunits and transcriptional repression of their target genes (Kawahara et al., 2009; Rothgiesser et al., 2010).

Other Types of Intercellular Communication

Beyond inflammation, accumulating evidence indicates that aging-related changes in one tissue can lead to aging-specific deterioration of other tissues, explaining the interorgan coordination of the aging phenotype. In addition to inflammatory cytokines, there are other examples of “contagious aging” or bystander effects in which senescent cells induce senescence in neighboring cells via gap-junction-mediated cell-cell contacts and processes involving ROS (Nelson et al., 2012). The microenvironment contributes to the age-related functional defects of CD4 T cells, as assessed by using an adoptive transfer model in mice (Lefebvre et al., 2012). Conversely, lifespan-extending manipulations targeting one single tissue can retard the aging process in other tissues (Durieux et al., 2011; Lavasani et al., 2012; Tomás-Loba et al., 2008).

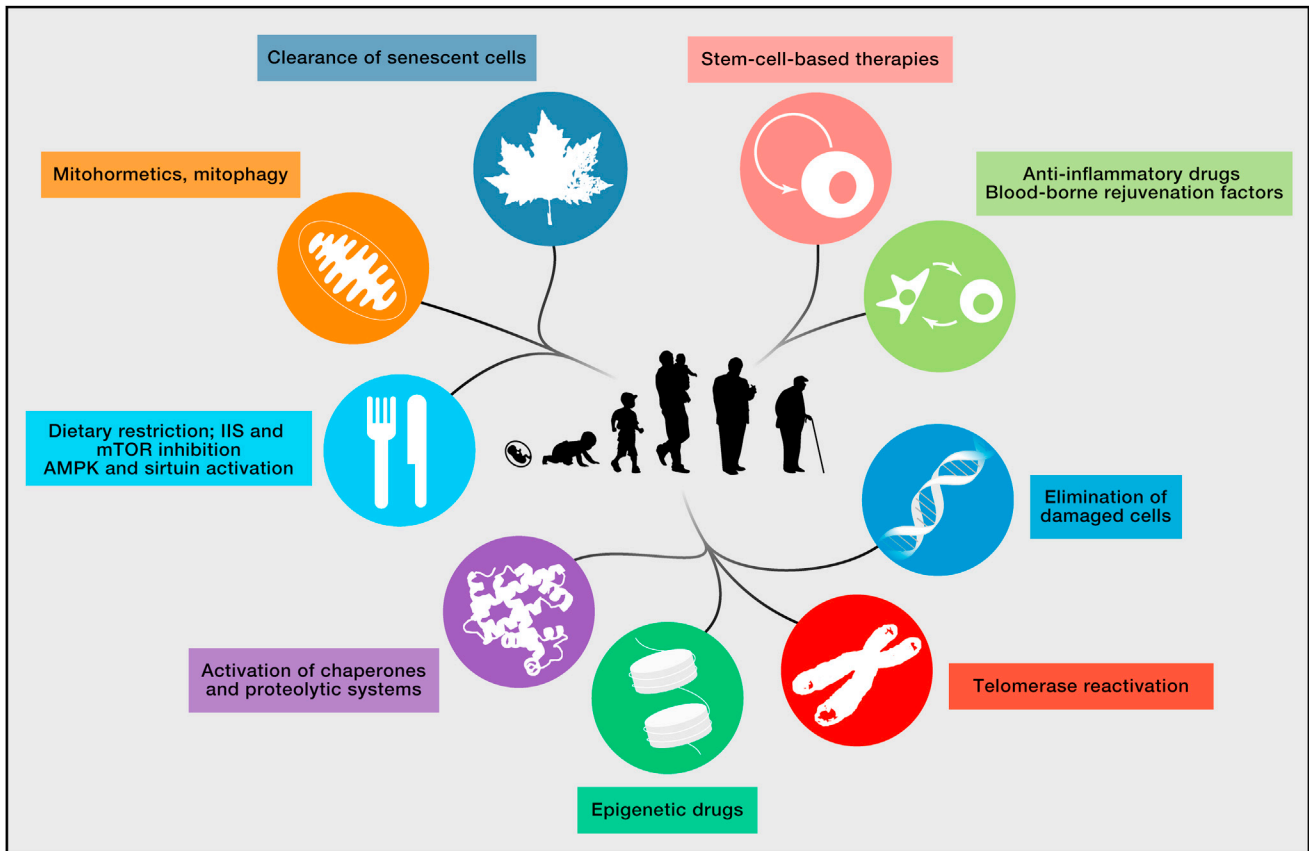


Figure 7. Interventions that Might Extend Human Healthspan

The nine hallmarks of aging are shown together with those therapeutic strategies for which there is proof of principle in mice.

Restoring Defective Intercellular Communication

There are several possibilities for restoring defective intercellular communication underlying aging processes, including genetic, nutritional, or pharmacological interventions that may improve the cell-cell communication properties that are lost with aging (Freije and López-Otín, 2012; Rando and Chang, 2012). Of special interest in this regard are the DR approaches to extend healthy lifespan (Piper et al., 2011; Sanchez-Roman et al., 2012) and the rejuvenation strategies based on the use of blood-borne systemic factors identified in parabiosis experiments (Conboy et al., 2005; Loffredo et al., 2013; Villeda et al., 2011). Moreover, the long-term administration of anti-inflammatory agents such as aspirin may increase longevity in mice and healthy aging in humans (Rothwell et al., 2011; Strong et al., 2008). Additionally, given that the gut microbiome shapes the function of the host immune system and exerts systemic metabolic effects, it appears possible to extend lifespan by manipulating the composition and functionality of the complex and dynamic intestinal bacterial ecosystem of the human body (Claesson et al., 2012; Ottaviani et al., 2011).

Overview

There is compelling evidence that aging is not an exclusively cell-biological phenomenon and that it is coupled to a general alteration in intercellular communication, offering opportunities to

modulate aging at this level. Excitingly, proof of principle exists for rejuvenation through blood-borne systemic factors (Conboy et al., 2005; Loffredo et al., 2013; Villeda et al., 2011).

Conclusions and Perspectives

A global view of the nine candidate hallmarks of aging enumerated in this Review suggests three categories: primary hallmarks, antagonistic hallmarks, and integrative hallmarks (Figure 6). The common characteristic of the primary hallmarks is the fact that they are all unequivocally negative. This is the case with DNA damage, including chromosomal aneuploidies; mitochondrial DNA mutations; and telomere loss, epigenetic drift, and defective proteostasis. In contrast to the primary hallmarks, antagonistic hallmarks have opposite effects depending on their intensity. At low levels, they mediate beneficial effects, but at high levels, they become deleterious. This is the case for senescence, which protects the organism from cancer but which, in excess, can promote aging. Similarly, ROS mediate cell signaling and survival but, at chronic high levels, can produce cellular damage; likewise, optimal nutrient sensing and anabolism are obviously important for survival but, in excess and during time, can become pathological. These hallmarks can be viewed as being designed for protecting the organism from damage or from nutrient scarcity. But when they are

exacerbated or chronic, they subvert their purpose and generate further damage. A third category comprises the integrative hallmarks—stem cell exhaustion and altered intercellular communication—that directly affect tissue homeostasis and function. Notwithstanding the interconnectedness between all hallmarks, we propose some degree of hierarchical relation between them (Figure 6). The primary hallmarks could be the initiating triggers whose damaging consequences progressively accumulate with time. The antagonistic hallmarks, being in principle beneficial, become progressively negative in a process that is partly promoted or accelerated by the primary hallmarks. Finally, the integrative hallmarks arise when the accumulated damage caused by the primary and antagonistic hallmarks cannot be compensated by tissue homeostatic mechanisms. Because the hallmarks co-occur during aging and are interconnected, understanding their exact causal network is an exciting challenge for future work.

Defining hallmarks of aging may contribute to (1) building a framework for future studies on the molecular mechanisms of aging and (2) designing interventions to improve human healthspan (Figure 7). However, there are still numerous challenges ahead in relation to understanding this complex biological process (Martin, 2011; Miller, 2012). The rapid development of next-generation sequencing technologies may have a special impact on aging research by facilitating the evaluation of the genetic and epigenetic changes specifically accumulated by individual cells in an aging organism (de Magalhães et al., 2010; Gundry and Vijg, 2012). These techniques are already being used to determine the whole-genome sequence of individuals with exceptional longevity, to perform comparative genomic studies between short-lived and long-lived animal species and strains, and to analyze age-associated epigenetic changes at maximum resolution (Heyn et al., 2012; Kim et al., 2011; Sebastiani et al., 2011). Parallel *in vivo* studies with gain- or loss-of-function animal models will be necessary for moving beyond correlative analyses and providing causal evidence in favor of the implication of these proposed hallmarks in the aging process. Besides the characterization of individual hallmarks, systems biology approaches will be required to understand the mechanistic links among the processes that accompany and lead to aging (Gems and Partridge, 2013; Kirkwood, 2008). Additionally, molecular analysis of the genome-environment interactions that modulate aging will help to identify drug targets for longevity promotion (de Magalhães et al., 2012). We surmise that ever more sophisticated approaches will eventually resolve many of the pending issues. Hopefully, these combined approaches will allow a detailed understanding of the mechanisms underlying the hallmarks of aging and will facilitate future interventions for improving human healthspan and longevity.

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