Cellular senescence and inflammation in aging and age-related disease

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CHAPTER 1

General introduction

GENERAL INTRODUCTION

1. AGING AND AGE-RELATED DISEASE

Introduction

Global human life expectancy has continuously and markedly increased over the last four decades and is projected to be 76 years in 2050 [1,2]. The number of people over the age of 60 as well as the percentage this group represents of the total population will dramatically grow over the next decades (Figure 1). This increase of life expectancy is accompanied with a longer period of work productivity and life experiences, often beyond the age of 60 and even stretching into ages of 70's and 80's. On the other hand, the rise of people experiencing age-related decline of their health and onset of frailty will impose a huge burden on the healthcare system and accompanying costs [2].

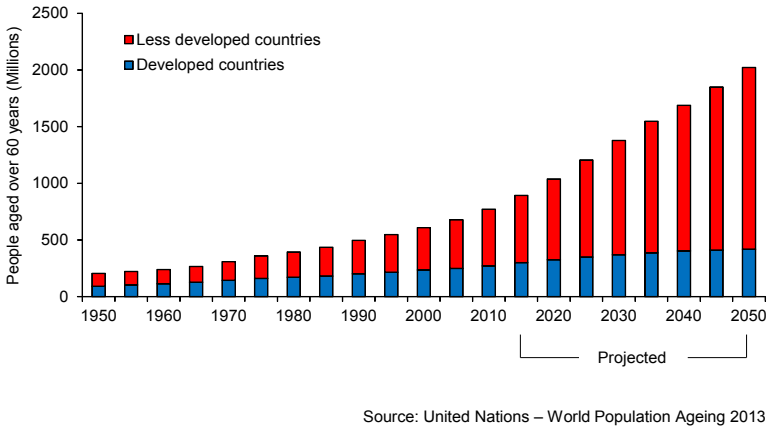

Figure 1. World Population Aging. Population aged 60 years or over by developmental region from 1950-2050. By 2050, the worldwide number of people aged over 60 years is projected to be more than five times that in 1950.

Aging is characterized by a gradual decline of physiological function over time that affects most living organisms. This degenerative process occurs at the molecular, cellular and organismal levels and is driven by various molecular, biochemical and metabolic alterations [2,3]. Traditional symptoms of aging in mammals include loss of skeletal muscle mass (sarcopenia), increased curvature of the spine (kyphosis), weight loss, greying and loss of hair, loss of eyesight and hearing, immune failure, loss of fertility, osteoporosis, loss of cognition, and many others [2]. Furthermore, aging is the main risk factor for major human

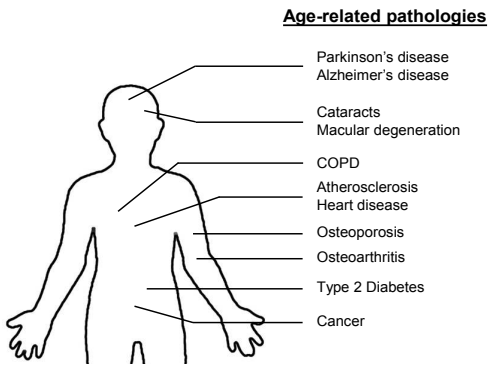

Figure 2. Major human pathologies with aging as the main risk factor.

pathologies, including cardiovascular diseases, type 2 diabetes, COPD, osteoarthritis, cancer, and neurodegenerative disorders (Figure 2) [4-8]. Better understanding of how aging increases the risk of these disorders will inform strategies to delay or prevent them.

Not long ago, aging was assumed as a passive haphazard process of deterioration, caused by the accumulation of multiple forms of damage in cells and tissues as a result of impaired cellular maintenance systems [4,9,10]. However, recent studies have revealed that a variety of interventions and pathways can control the rate of aging [10]. Many of these pathways were first identified by gene mutagenesis studies in small, short-lived organisms, but a substantial portion of these pathways has also been shown to extend lifespan in mammals. In spite of this, the molecular mechanisms that underlie these interventions remain poorly understood, further illustrating the extreme complexity of the aging process [9,10]. A number of theories and/or cellular mechanisms have been proposed to explain the biology of aging, including cellular senescence, inflammation, deregulated nutrient-sensing, genomic and epigenomic alterations, loss of protein homeostasis, mitochondrial dysfunction, stem cell depletion or dysfunction, and the altered immune system (Figure 3) [5,9,11]. In this thesis, I will focus on the role of cellular senescence (Section 2 and 3) and inflammation (Section 5) in aging and age-related disease. In addition, I will shortly highlight the other pathways involved in the aging process.

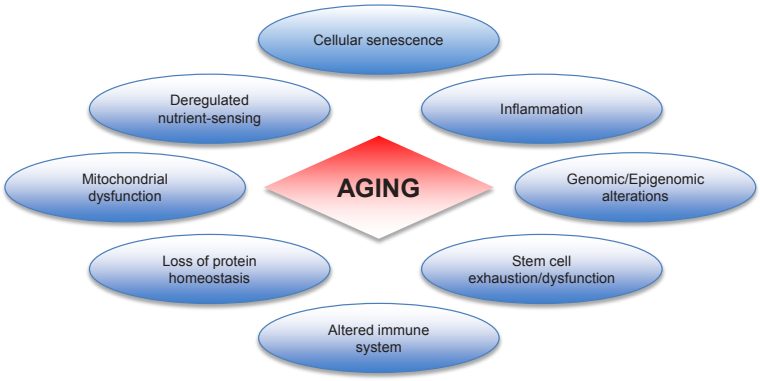

Figure 3. Hallmarks of Aging. This scheme depicts the cellular and molecular mechanisms that contribute to the process of aging.

Nutrient-sensing pathways

The insulin- and insulin-like growth factor 1 (ISS) signaling pathway, which participates in glucose sensing, was identified as the first pathway that can influence aging. Mutations and genetic polymorphisms that affect the functionality of key components of the ISS pathway extend lifespan in a number of model organisms and humans [9,11,12]. In addition, downstream effectors of the ISS pathway and other related nutrient-sensing systems have also been associated with longevity. This includes variants of the forkhead box protein O (FOXO) transcription factor family causing higher transcriptional activity [11,13], and increased activity of adenosine monophosphate-activated protein kinase (AMPK) [14-16]. Another established example of life extension is the genetic or pharmacological inhibition of the mammalian target of rapamycin (mTOR) pathway [17-19]. In contrast, high fat diet feeding in flies and mice hyperactivates mTOR and hereby contributes to two well known age-related disorders namely insulin resistance and obesity [20,21]. Consistent with the genetic or pharmacological interventions that cause reduced signaling of the nutrient-sensing

pathways, it was demonstrated that dietary restriction extends lifespan in various organisms, including mice and nonhuman primates (Figure 4) [11,22-24].

Altogether, current studies support the notion that decreased insulin nutrient-sensing signaling extends longevity, while overnutrition accelerates aging [5].

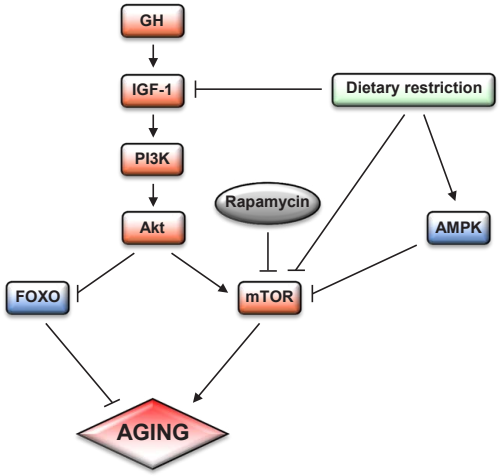

Figure 4. Deregulated nutrient-sensing pathway. Overview of the nutrient-sensing pathway involving the growth hormone (GH) and the insulin growth factor 1 (IGF-1) signaling pathway. An increase in GH signaling leads to activation of the signaling cascade IGF-1, phosphatidylinositide 3-kinase (PI3K) and Akt pathways. Subsequently, Akt either inhibits FOXO or activates mTOR signaling, leading to accelerated aging. In addition, two interventions that delay aging, namely dietary restriction and rapamycin, are depicted as well as their modes of action.

Genomic damage and epigenomic alterations

Genomic DNA damage accumulates in cells from various organisms and humans during chronological aging [5]. Throughout life, DNA is constantly being damaged by spontaneous hydrolytic metabolites, environmental factors and enzymatic reaction byproducts, like reactive oxygen species (ROS) [25]. To safeguard against the high frequency and great variety of genomic DNA lesions, organisms have developed a complex network of DNA repair mechanisms [26]. One of the key pathways is the DNA damage response (DDR) pathway, a signaling cascade in which ATM/ATR kinases inhibit cell cycle progression through stabilization of p53 and transcriptional activation of p21. Mutations in genes involved in the DDR have been reported in patients with premature aging (progeroid) syndromes such as Cockayne's syndrome and thrichotiodystrophy [25]. Furthermore, mouse models of nucleotide-excision repair syndromes demonstrate a strong correlation between the degree to which the DDR pathways are malfunctioning and the severity of accelerated aging, suggesting a causal relationship [25].

At the chromosomal level, both structural and numerical changes occur with aging. For instance, in mice, age-related aneuploidization has been observed in various tissues, including brain, spleen and colon [27-29]. In humans, aneuploidy was augmented in the cerebral cortex of Alzheimer's disease and ataxia telangiectasia patients [30]. Furthermore, increased clonal mosaicism for large chromosomal anomalies were detected during aging and in the etiology of cancer [31,32].

Continuous epigenetic alterations occur from early adulthood on, including variations in DNA methylation patterns, modifications of histones and chromatin remodeling [5,33]. Increased histone methylation is linked to aging in invertebrates [34]. Although, it is unclear whether histone-modifying enzymes influence aging directly through epigenetic alterations, altered transcription, or both [5]. Sirtuins are a class of NAD^+-dependent deacetylases, which deacetylate both histones and proteins [35]. Overexpression of certain sirtuin genes in mammals delays various features of aging and extends lifespan [35,36], suggesting that manipulating the epigenetic landscape holds promise as a therapeutic target for treating age-related diseases and extending healthy lifespan.

Loss of protein homeostasis

Protein homeostasis is the concept of complex and integrated biological pathways that control the balance between protein synthesis, folding, trafficking, aggregation and degradation within and outside the cell [37]. In order to preserve the stability and functionality of proteins, cells have evolved a number of quality control systems. Chaperones maintain the stability and facilitate the correct folding of proteins, while the autophagy-lysosomal system and the ubiquitin-proteasome system ensure proper degradation of proteins [38-40]. Aging and age-related disorders have been associated with compromised protein homeostasis. For instance, misfolded and/or aggregated proteins contribute to the development of age-related disorders, like Parkinsons's disease, Alzheimer's diseases and cataracts [37]. Furthermore, deficiency of co-chaperone proteins, such as the carboxyl terminus of Hsp70-interacting protein (CHIP) in mice markedly reduced lifespan with accelerated age-related pathologies [41]. Decline of the autophagy-lysosomal system and the ubiquitin-proteasome system also occurs during aging [42,43]. Induction of autophagy and upregulation of components of the ubiquitin-proteasome system can promote longevity in yeast and invertebrates [44-46]. Conversely, studies in mammalian organisms that demonstrate that improved protein homeostasis can delay aging are still lacking.

Mitochondrial dysfunction

The mitochondrial free radical theory of aging was proposed by Denham Harman and postulates that the progressive decline in mitochondrial function with aging accumulates free radicals, such as ROS, which subsequently causes damage to macromolecules and accelerates aging [47]. Early studies confirmed that ROS accelerates aging, but recent work demonstrating that increased ROS levels can prolong lifespan suggest that the relationship between ROS and aging is more complex [48,49]. With evidence that ROS can have either positive, negative or neutral effects on aging, the free radical concept of aging clearly needs re-evaluation [50-53].

As mitochondria age they become progressively less efficient and might even have toxic effects. The reduced efficiency of the respiratory chain may result from multiple mechanisms, including reduced biogenesis, impaired removal of damaged mitochondria by autophagy and the accumulation of mutations and deletions of mitochondrial DNA [5,54]. Acute damage by for instance free radicals can activate the permeabilization of the mitochondrial membrane to induce apoptosis or necrosis and pro-inflammatory signaling [54]. Along this line, genetic impairment of mitochondrial function accelerates aging in mice by engaging apoptotic signaling [52,53,55].

Stem cell depletion or dysfunction

A decline in the capacity to maintain homeostasis or repair tissues is a hallmark of aging and is accompanied with depletion or impaired function of various stem cell populations [56,57]. Studies in aged mice have demonstrated diminished regenerative potential in various tissues, including skeletal and cardiac muscle, and hematopoietic system [57-59]. The underlying mechanisms seem complex and diverse and include accumulation of DNA damage and telomere attrition leading to upregulation of cell cycle inhibitors [57,60]. On the other hand, increased growth factor signaling in aged muscle stem cells results in loss of quiescence, muscle stem cell depletion and diminished regenerative capacity [61].

Recent studies have revealed the importance of cell extrinsic pathways in the decline of stem cell function. Transplantation of muscle-derived stem cells from young mice to progeroid mice extends lifespan and improves degenerative changes in other tissues possibly by secreted factors. This was confirmed when young muscle-derived stem cells were able to rescue proliferation and differentiation defects of old muscle-derived stem cells upon co-culturing [62]. In addition, parabiotic pairing of young and old mice have proven that systemic factors from young mice can reverse the age-related decline of muscle stem cell function [63].

2. CELLULAR SENESCENCE

Introduction

Cellular senescence can be defined as the irreversible arrest of cell proliferation and is accompanied with characteristic phenotypic alterations, when cells are exposed to a certain stress [3,64,65]. Senescence was first described in 1961 when Hayflick and Moorhead showed that primary human cells have a finite replicative lifespan and undergo replicative senescence after extensive serial cultivation. The number of divisions a cell can complete upon reaching the end of their replicative lifespan is known as the Hayflick limit [66]. Along this line, it was observed that telomeres of diploid cells shorten with every cell division, and ultimately become critically short, which causes replicative senescence [3,67]. Human cells stably expressing telomerase can maintain their telomere length, bypass their Hayflick limit and avoid replicative senescence [67,68]. This, together with the observation that telomerase is expressed in immortalized human cell lines and in most human tumors, suggests that senescence has evolved a tumor suppressor program [67]. Consistent, expression of oncogenic *ras* in primary human or rodent cells leads to a permanent G1 arrest (oncogene-induced senescence) [69]. Next to cessation of cell proliferation, senescent cells undergo a wide variety of distinctive phenotypic changes in chromatin organization and gene expression. These alterations comprise the production and secretion of pro-inflammatory cytokines, chemokines, and proteases, a phenomenon that is known as the senescence-associated secretory phenotype (SASP) [3,64,65,70]. The nature of these secreted factors suggests that senescence might be involved in many other biological processes, and is not solely a mechanism for preventing cancer. Recent studies have indicated that cellular senescence also plays a role in embryonic development [71,72], tissue repair [73], wound healing [74] and aging [75].

Inducers of senescence and tumor suppressor pathways

The causes and underlying mechanisms that induce cellular senescence are still not well understood, but *in vitro* studies have identified a number of stresses and effector pathways, which will be discussed in this section (Figure 5). As mentioned before, telomere attrition was the first mechanism to be associated with the induction of senescence [67,68]. Telomeres are a region of repetitive nucleotide sequences at the end of chromosomes that cannot be completely replicated by polymerases. In most human cells, which lack telomerase, these telomeres will shorten after every cell division [67]. Repeated cell division will eventually result in critically short and dysfunctional telomeres that provoke the DDR, which blocks cell cycle progression [76-78]. Dysfunctional telomeres are not reparable and cause a persistent DDR, hereby enforcing the cell cycle arrest [65,78,79]. Other potential tumor stresses that can provoke the senescence response are genomic DNA damage, like DNA double-strand

breaks (DSB) and other lesions caused by either aging, radiation, cytotoxic chemotherapies, oxidative stress, or other agents [80-84]. As with telomere attrition, severe DNA lesions cause persistent DDR signaling, while mild DNA damage only generates a temporary growth arrest and transient DDR signaling [3,78,80]. Oncogenes can also elicit senescence. Activation of oncogenes, like oncogenic H-RasV12 in human cells provokes hyper-proliferation, which causes DNA replication errors and formation of DSBs leading to activation of the DDR [85]. On the other hand, inhibition of oncogenic c-Myc perturbs chromatin organization, which is DDR-independent and induces senescence via activation of the $p16^{Ink4a}$ pathway [86]. Strong proliferation-associated signals that act via overexpression of mitogen-activated protein-kinase (MAPK) pathway, activation of growth factors, and chronic stimulation of cytokines can all provoke the senescence response as a tumor protective mechanism [3,65]. Furthermore, inactivation of a number of tumor suppressors, like RB and PTEN, results in a senescence growth arrest [3,65,87].

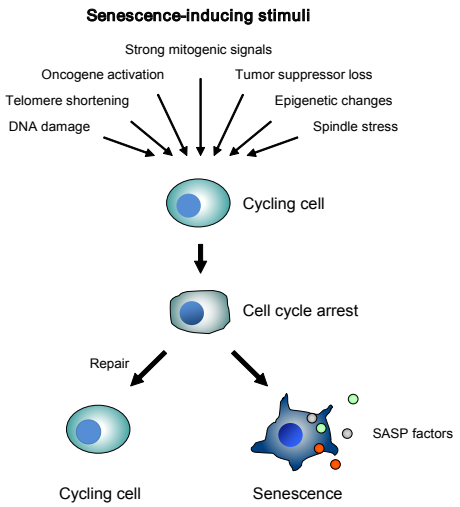

Figure 5. Senescence-activating stressors. A variety of stressors can provoke the cellular senescence response and induce a temporal cell-cycle arrest. Upon mild stress, temporary arrested cells may be successfully repaired and possibly will resume normal cell proliferation. In case of severe stress/damage, arrested cells transition to a senescent growth arrest and undergo a wide variety of distinctive phenotypic changes, including morphological alterations and the secretion of SASP components.

Alteration in the epigenome is one of the characteristics of cellular senescence, including the formation of heterochromatin that can cause repression of certain loci [88]. In contrast, perturbations of chromatin organization can also induce gene expression that provokes senescence. For example, histone deacetylase inhibitors can induce global relaxation of the chromatin, resulting in derepression of $p16^{Ink4a}$ [89].

Last, spindle stress caused by insufficiency of the mitotic checkpoint proteins BubR1, Bub3 and Rae1 in mouse embryonic fibroblasts (MEFs) induce the expression of proteins in p53/p21 and $p16^{Ink4a}$/RB pathway resulting in accelerated senescence [90,91].

The senescence-inducing stimulus determines whether either or both of p53/p21 and $p16^{Ink4a}$/RB tumor suppressor pathways are engaged in the initial growth arrest and in senescence maintenance (Figure 6) [92-95]. Both pathways are highly complex, regulate each other and mainly control the senescence response [3,93,96,97].

Characteristics of senescent cells

Senescent cells are defined by their permanent cell cycle arrest and a number of other features and markers can be used to identify a senescent cell (Table 1). However, like arrested cell growth, many of these features and markers are not unique to senescent cells. Furthermore, senescent cells are heterogeneous meaning that not all cells express the same genes and contain the same set of traits [3].

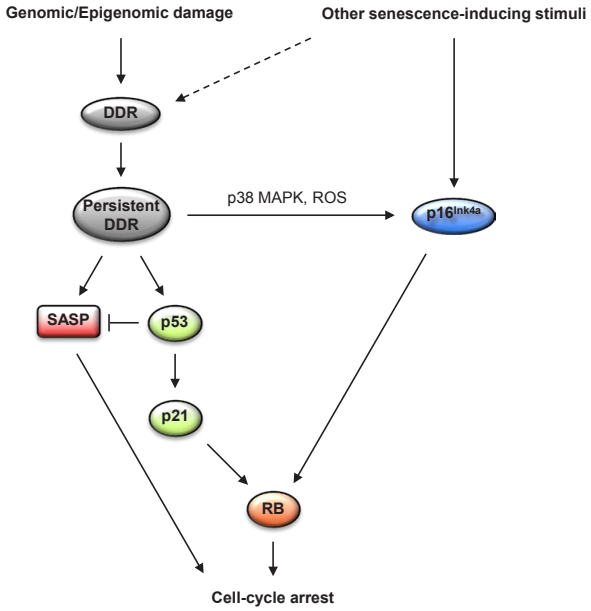

Figure 6. Main effector pathways of the senescence program. The senescence-inducing stressor governs whether either or both the p53/p21 and $p16^{Ink4a}$/RB tumor suppressor pathways are engaged in the senescence response. Genomic damage activates the DDR response, which can ultimately become chronic or persistent leading to increased expression of $p16^{Ink4a}$ through enhanced activation of p38 MAPK and higher ROS levels. Other senescence-inducing stimuli that not directly entail genomic or epigenomic damage can induce the $p16^{Ink4a}$ suppressor and in some instances indirectly engage the DDR (dashed line). $p16^{Ink4a}$ triggers the activation of the RB tumor suppressor, which silences certain proliferative genes via heterochromatin formation resulting in the senescence growth arrest. Persistent or chronic DDR induces the SASP and activates the p53 tumor suppressor. p53 can induce a growth arrest via the activation of downstream p21 and RB, while SASP components can also directly stimulate the senescent growth arrest.

Table 1. Features and markers of senescent cells

Features
Loss of proliferative potential
Enlarged and flattened morphology
Senescence-associated β-galactosidase activity
Senescence-associated heterochromatin foci
Altered gene expression
Telomere-dysfunction-induced foci
DNA segments with chromatin alterations reinforcing senescence
Senescence-associated secretory phenotype
Molecular markers
Increased p53 and p21 expression
Increased p16 expression
Lamin B1 reduction

Cellular senescence occurs after prolonged inhibition of Cdk-cyclin activity by p21 or $p16^{Ink4a}$, or both, and the switch to continuous expression of p53 [65,98]. The tumor suppressor protein $p16^{Ink4a}$ is now regularly used as a marker for senescence. Expression of $p16^{Ink4a}$ is low in most cells and tissues under normal circumstances and is upregulated in cells upon exposure to various senescence-inducing stimuli [99,100]. In addition, $p16^{Ink4a}$ expression is gradually induced in cells and tissues of various organisms during aging [101-104]. Another feature of senescent cells is that they are generally enlarged up to two times of the volume of normal cells and have a flattened morphology. One the first widely used markers for senescence is the histochemical staining for senescence-associated β-galactosidase (SA-βgal) [105]. Overexpression and accumulation of the endogenous lysosmal β-galactosidase activity in senescent cells can be easily detected at near-neutral pH [106].

Senescence activation by either the p53/p21 pathway or the $p16^{Ink4a}$/RB tumor suppressor pathways leads to downregulation of nuclear lamin B1 expression [107,108]. Reduced lamin B1 levels are a key trigger for global and local chromatin remodeling that affects gene expression and hereby reinforces the senescence response [109]. Many cells that

undergo senescence show formation of senescence-associated heterochromatin foci (SAHF), which are enriched in chromatin modifications and can possibly prevent the activation of proliferative-associated genes by mitogenic signals [88,110]. All these senescence-associated alterations in the chromatin landscape result in major modifications in gene expression [109,111,112]. Many of the senescence-inducing stimuli cause genomic damage, resulting in persistent DNA damage foci and activation of the DDR pathway [3]. Examples of genomic damage-related markers are telomere dysfunction-induced foci (TIF) and DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS) [104,113]. Both are containing p53 binding protein (53BP1) foci, distinct from the foci that form immediately after DNA damage [3].

Senescence-associated secretory phenotype

One of the most intriguing characteristics of senescent cells is the senescence-associated secretory phenotype (SASP). The great variety of the SASP components can explain the contributing role of cellular senescence in a number of biological processes [92,114]. The SASP entails the secretion of numerous pro-inflammatory cytokines and chemokines, growth factors and proteases [65,94,115]. They can include IL-1, IL-6, IL-8, GROα, monocyte chemo-attractant proteins (MCPs), macrophage inflammatory proteins (MIPs), granulocyte macrophage colony-stimulating factor (GM-CSF), and various metalloproteinases [3,115]. These secreted factors can either have beneficial or detrimental effects depending on the context (see below). Many of the SASP features are conserved between mouse and human cells. Secretion of SASP factors occurs in a wide variety of cells, including fibroblasts, epithelial cells, endothelial cells and astrocytes, and also takes place *in vivo* in mice and humans [114].

Cells that undergo senescence due to genomic damage or epigenomic alterations show a SASP phenotype. However, cells that senesce due to overexpression of the tumor suppressors p21 or $p16^{Ink4a}$ display a growth arrest together with some other characteristics of senescence, but express no SASP [3,116]. Inducers of the SASP include dysfunctional telomeres, DNA damage, mitogenic signals, epigenetic changes, oxidative stress and other senescence-causing stimuli (Figure 7). The SASP phenotype can show great variety in the secretion of components depending on the cell types, the strength of and the kind of senescence-inducing stimuli [3,78,94,115,117,118]. Rapid activation of DDR signaling after DNA damage is not sufficient to induce the SASP. In many cases, SASP activation requires persistent DDR signaling via the DDR proteins ataxia telangiectasia mutated (ATM) and checkpoint kinase 2 (CHK2) and is a slow and gradual process [94,115]. How signaling via the DDR pathway can promote the expression of various SASP components is incompletely understood, but a possible mechanism involves chromatin remodeling which leads to profound changes in transcriptional regulation [109,112]. This is supported by the fact that senescent cells continuously undergo genomic and epigenomic alterations, including increased transcription of transposable elements and chromatin budding that result in the formation of cytoplasmic chromatin fragments [65]. Another positive regulator of SASP is the nuclear factor kappaB (NF-κB) pathway, which controls inflammatory cytokine gene expression [119]. Proteomics analysis of senescent chromatin revealed that NF-κB subunit p65 as the main transcription factor that accumulates on chromatin of senescent cells and acts as a major regulator of the SASP by influencing the expression of numerous genes [120]. In contrast, functional loss of the p53 tumor suppressor protein markedly accelerated and amplified the development of SASP, suggesting that p53 normally restrains the SASP

(Figure 6) [115]. Recent studies have demonstrated that several senescence-inducing stimuli can produce SASP components without DNA damage, indicating that there are also DDR-independent mechanisms [71,72,121].

SASP and its impact on biological processes

Due to the complexity of the SASP, it was obvious that senescent cells are implicated in various biological processes that engage paracrine signaling, such as cell proliferation, angiogenesis, epithelial-to-mesenchymal transition, inflammation, stem cell renewal and differentiation, wound healing and tissue repair [63,73,74,122-125]. In addition, several SASP components including WNT16B, IL-6, IL-8 and growth-regulated oncogene 1 (Gro-1) are necessary for the onset and maintenance of the senescence growth arrest [118,126,127]. Recent studies have suggested that the nature of processes in which senescence has been implicated may require different courses of action that can be divided in acute versus chronic senescence (Figure 7) [65]. An example of acute senescence is the process of wound healing. The matricellular protein CCN1 is dynamically expressed at sites of wound repair and induces senescence of myofibroblasts through integrin-dependent ROS generation by activation of NOX1. CCN1-induced senescence of myofibroblasts limits excessive fibrosis in cutaneous wound healing by inducing the expression of anti-fibrotic genes [74]. In addition, chemical-induced liver fibrosis in mice will eventually induce senescence in activated hepatic stellate cells to prevent excessive liver fibrosis. Senescent stellate cells exhibit enhanced secretion of extracellular matrix-degrading enzymes and increased immune surveillance. Subsequently, natural killer cells eliminate senescent stellate cells and hereby facilitate the resolution of liver fibrosis, indicating that the senescence program limits fibrosis during acute tissue damage [73]. Senescence also occurs during embryonic development at multiple locations to allow tissue alteration. This is a highly programmed developmental response that includes the induction of p21 and activation of downstream signaling pathways followed by macrophage infiltration, clearance of senescent cells, and tissue remodeling [71,72].

Accumulation of macromolecular damage in cells over time will result in an increasing number of cells undergoing chronic senescence, which is uncontrolled and stochastic in nature. Chronic senescence may be one of the underlying mechanisms that play a role in the development of aging and age-related diseases (see next section), and cancer, but direct causal evidence is lacking thus far (Figure 7).

Senescence is a tumor-suppressive program that prevents the proliferation of damaged cells, but there is mounting evidence that senescent cells can also drive tumor progression. Xenograft studies have demonstrated that co-injection of senescent cells can promote proliferation of epithelial tumor cells, stimulate tumor invasion and promote tumor angiogenesis in immunocompromised mice, partially due to secretion of SASP components [122,128,129]. Furthermore, senescent fibroblasts promote epithelial-to-mesenchymal transition in premalignant epithelial cells, a critical step in the development towards a metastatic cancer, through production of SASP factors IL-6 and IL-8 [115,123,130]. SASP elements may also stimulate tumor initiation by causing chronic tissue inflammation and impaired functioning of immune cells [124,131]. The overall picture is that accumulation of senescent cells may create a microenvironment that allows the development and the progression of cancer. However, this process is not straightforward as the same SASP factors may promote tumor progression in one setting, but can be essential in tumor suppression in the other [3].

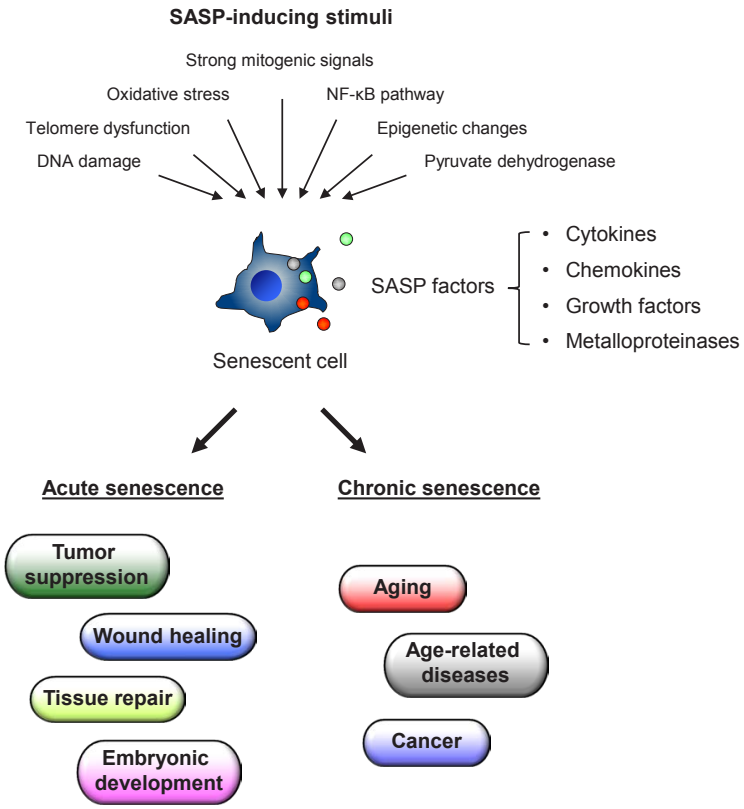

Figure 7. Senescence-associated secretory phenotype implicates many biological processes. Numerous stresses and stimuli can induce SASP, which can show great variety in the secretion of components, including cytokines, chemokines, growth factors and metalloproteinases. Due to the great diversity of the secreted SASP components, SASP has been implicated in a number of biological processes, such as tumor suppression, wound healing, tissue repair, and embryonic development, but also in tumor promotion, aging and age-related diseases.

3. SENESCENCE IN AGING AND AGE-RELATED DISEASE

Accumulation of senescent cells during aging and age-related diseases

Hayflick and Moorhead already interpreted the phenomenon of replicative senescence as one of the fundamental mechanisms underlying the process of organismal aging [66]. Consistent with this, it was demonstrated that senescent cells accumulate with aging *in vitro*, and in a number of tissues in aged mice and aged human skin [101,105]. By now it is widely accepted that senescent cells increase with age in tissues of humans, primates, and rodents [81,104,132-134]. Furthermore, accumulation of senescent cells has been observed at sites of various age-related diseases including cardiovascular disease [135,136], osteoporosis [137], arthritis [138,139], and neurodegenerative disorders [140,141]. This increase in senescent cells over time can be a cause of accumulation of DNA damage and/or the increase of other senescence-inducing stimuli. Senescing human cells and aging mice show enhanced numbers of DNA lesions with unrepairable DSBs [80,81]. Another possibility is that clearance of senescent cells by the immune system is less efficient with aging. Elimination of senescent cells by

the immune system was nicely demonstrated in a model of liver fibrosis and liver carcinoma [73,142]. Senescent hepatic stellate cells augment the expression of immune modulators encoding cytokines or receptors that activate natural killer cell function [73]. This suggests that senescent cells have a self-elimination program (designated as 'senescence surveillance') that acts via attracting and activating various immune cells by the secretion of inflammatory cytokines and chemokines [65,73,131,142]. With aging, efficient elimination of senescent cells is likely reduced as a result of aging-associated immune deficiency [143]. Studies have demonstrated that the function of hematopoietic stem cells declines with aging, which results in impaired lymphopoiesis and increased myelopoiesis, leading to impaired immune function [144]. Furthermore, the immune system is unable to maintain a balanced T-cell repertoire due to changes in T-cell production and consumption in the later stages of life of mammals [145].

Senescence and aging

The relevance of *in vivo* senescence in the development of aging and age-related diseases has initially focused on the Cdkn2a locus, which encodes two tumor suppressors, namely $p16^{Ink4a}$ and $p19^{Arf}$. Both $p16^{Ink4a}$ and $p19^{Arf}$ can induce senescence in cultured cells and the level of both proteins increases with aging in many tissues [75,101,146]. Testing whether the induction of $p16^{Ink4a}$ and $p19^{Arf}$ causes *in vivo* senescence and organismal aging was hampered because mice deficient for $p16^{Ink4a}$ and $p19^{Arf}$ die early from cancer before they reach the age that normal mice start to show age-related phenotypes [92,147]. However, genetic inactivation of $p16^{Ink4a}$ in a BubR1 progeroid mouse model attenuates both cellular senescence and premature aging in certain tissues. In contrast, inactivation of $p19^{Arf}$ exacerbates senescence and aging in BubR1 mutant mice. This study demonstrates for the first time that senescent cells can promote age-related phenotypes [75].

SASP and aging

It is however not clear how senescence can drive aging. One possibility is that senescence contributes to the decline of the regenerative capacity that occurs with aging by altering the systemic environment through the secretion of various SASP factors [65,125]. Conversely, parabiotic pairings of old and young mice proved that systemic factors from young mice could restore the proliferation and regenerative capacity of aged satellite cells [58].

Chronic inflammation is associated with aging and plays a causative role in numerous age-related disorders, like atherosclerosis, diabetes and cancer (see section inflammation) [124]. With aging and at these sites of age-related diseases, senescent cells can accumulate and secrete various pro-inflammatory cytokines and chemokines to promote infiltration of macrophages and lymphocytes, and hereby potentially induce or accelerate a state of chronic inflammation.

Another scenario that can accelerate age-related tissue deterioration is by paracrine senescence. In two studies it was demonstrated that cells undergoing oncogene-induced senescence were able to transmit senescence to healthy neighboring cells via the secretion of multiple SASP components [148,149].

Furthermore, senescent cell secretion of proteases can possibly disrupt tissue structure and organization by cleavage of extracellular matrix proteins or other components of the tissue microenvironment [115,130]. This environment then allows epithelial-mesenchymal transition and stimulates the invasiveness and metastatic properties of epithelial cells [115,130].

4. BUBR1 IN CANCER AND AGING

Mitotic checkpoint protein

Budding uninhibited by benzimidazole-related 1 (BubR1) is a mitotic checkpoint protein that is considered to be the mammalian ortholog of yeast mitotic-arrest deficient 3 (Mad3) [150,151]. *BubR1* encodes a 1052 amino acid serine/threonine kinase and contains a number of functional domains (Figure 8) [150,152-156]. BubR1 protein levels are continuously changing throughout the different phases of the cell cycle. During most of the cell cycle BubR1 protein levels are extremely low, but they rapidly rise during G2/M phase [157].

Figure 8. Schematic overview of mouse BubR1 protein. Functional domains of BubR1 are indicated: two N-terminal KEN-boxes, destruction box-motifs associated with APC/C^{Cdc20} inhibition (also known as Cdc20 binding domain 1); a tetratricopeptide (TPR) motif for binding to Knl1; a Gle2-binding-sequence (GLEBS) motif for Bub3 binding and kinetochore localization; Cdc20 BD2, C-terminal Cdc20 binding domain 2; and a kinase domain.

BubR1 is a core component of a multi-protein network known as the mitotic checkpoint complex or the spindle assembly checkpoint. This cellular maintenance system of the eukaryotic cell cycle ensures accurate chromosome segregation by stalling anaphase onset until are chromosomes are properly attached to the mitotic spindle [158]. BubR1 inhibits the activity of the anaphase promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase through binding to its co-activator subunit cell division cycle 20 (Cdc20) (Figure 9, left). [159]. Upon proper bi-orientation of all chromosomes, BubR1 detaches from Cdc20 resulting in the polyubiquitination of securin and cyclin B1, which are inhibitors of separase (Figure 9, middle). Separase-mediated cleavage of cohesion rings that holds duplicated sister chromatids together then initiates anaphase (Figure 9, right) [160]. Furthermore, depletion of BubR1 in human cells revealed a function of BubR1 in stabilization of microtubule-kinetochore attachments [160].

Low levels of BubR1 protein cause a compromised function of the mitotic checkpoint. For instance, haplo-insufficiency of BubR1 in mouse embryonic fibroblasts (MEFs) resulted in an average BubR1 protein level of about 25% of that in wild-type MEFs. *BubR1*$^{+/-}$ MEFs contain reduced amount of securin and Cdc20 and increased numbers of spontaneous formed micronuclei, both hallmarks of a compromised spindle checkpoint [161]. Gradual reduction of BubR1 protein levels in MEFs demonstrated that a certain threshold of BubR1 protein is necessary to maintain proper mitotic checkpoint control. *BubR1*$^{+/-}$ MEFs could induce a sustained pre-anaphase arrest in the presence of the spindle poison nocodazole, indicating that the spindle assembly checkpoint is intact. Conversely, in *BubR1* MEFs that contain two hypomorphic alleles (*BubR1*$^{H/H}$), which have about 10% of wild-type BubR1 protein, a significant smaller percentage of the cells were able to arrest, indicating that spindle assembly checkpoint was severely compromised. *BubR1*$^{H/H}$ MEFs were also unable to maintain cyclin B-associated Cdc2 kinase activity after nocodazole release [90]. Furthermore, low levels of BubR1 increased the incidence of premature sister chromatid separation (PMSCS) and anaphase figures with lagging chromosomes, both hallmarks of defective spindle assembly checkpoint [90].

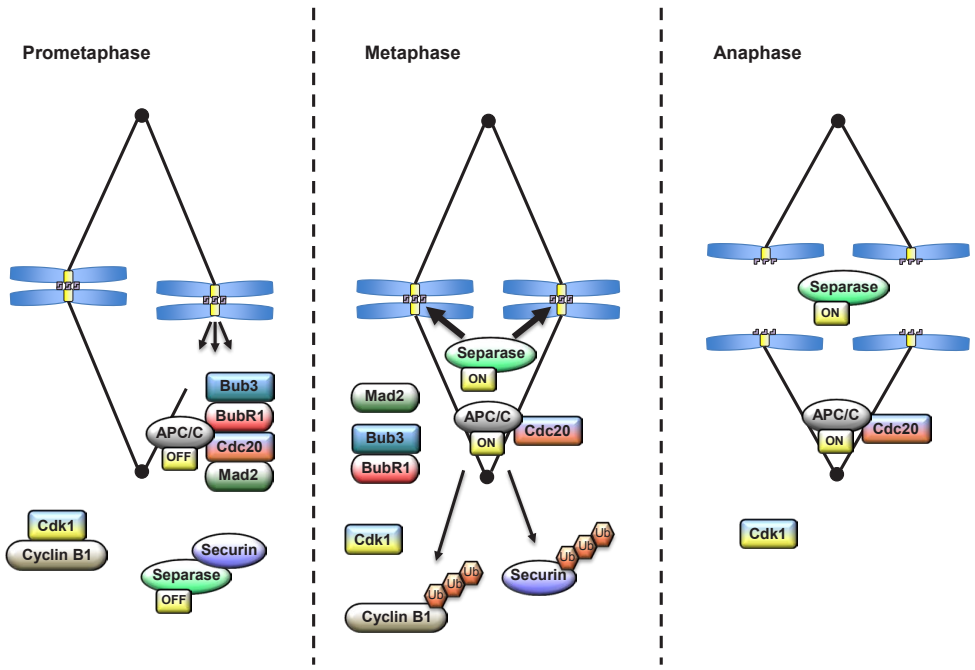

Figure 9. Simplified model of APC/C regulation by the mitotic checkpoint complex. In early mitosis (prometaphase), not all chromosomes are properly attached to the mitotic spindle. This triggers the recruitment of the mitotic checkpoint proteins BubR1 and Bub3 to associate with Mad2 and Cdc20. The association of BubR1 with Bub3, Mad2 and Cdc20 is known as the mitotic checkpoint complex, which interacts with APC/C leading to its inactivation (left). In metaphase, all chromosomes undergo bipolar attachment and are aligned along the metaphase plate, resulting in dissociation of the mitotic checkpoint complex and activation of the APC/C-Cdc20. Activated APC/C-Cdc20 polyubiquitinates cyclin B1 and securin, which are both inhibitors of separase (middle). Subsequently, separase mediates the cleavage of cohesin rings at the centromeres and chromosome arms, allowing chromosome separation and mitotic progression (right).

BubR1 and aneuploidy

Inaccurate segregation of whole chromosomes can result in abnormal number of chromosomes in the daughter cells, a phenomenon known as aneuploidy [162]. Aneuploidy in humans is associated with severe developmental abnormalities and pathologies of disease, including Down syndrome [163]. In addition, aneuploidy in embryos is the leading cause of congenital birth defects and miscarriages [164].

Murine BubR1 deficiency results in embryonic lethality at day 8.5 in utero due to massive apoptosis. Furthermore, *BubR1*$^{-/-}$ blastocysts demonstrated impaired proliferation and increased atrophy [165]. Gradual reduction of BubR1 protein levels causes increased rates of aneuploid metaphases in MEFs as expected in cells with defective spindle assembly checkpoint. Karyotype analysis on metaphase spreads *BubR1*$^{H/H}$ MEFs showed increased numerical abnormalities compared to wild-type and *BubR1*$^{+/-}$ MEFs [90]. *BubR1*$^{-/H}$ pups showed significant aneuploidy at birth, while *BubR1*$^{H/H}$ pups did not. However, at 2 months of age *BubR1*$^{H/H}$ mice have developed mild aneuploidy that increased in degree and severity with aging [90]. Reduced expression of BubR1 also induced mild aneuploidy in spermatocytes and oocytes, which might account for the infertility in both male and female *BubR1*$^{H/H}$ mice [90].

BubR1 and cancer

Aneuploidy is also a remarkable feature of human cancer, with numerous animal models making it clear that aneuploidy can predispose to tumor development [166,167]. However, mutations in mitotic checkpoint proteins are relatively uncommon in most human cancers or cancer lines. For *BUBR1* a number of mutations have been reported: analysis of 19 selected colorectal cancer cell lines revealed *BUBR1* missense mutations in two colorectal cancer cell lines, with one mutation predicted to remove part of the kinase domain [168]. Mutations and deletions of human *BUBR1* were detected in three out of ten primary adult T-cell leukemia/lymphomas (ATTL), including two missense mutations, and one with a small heterozygous deletion [169]. Deep sequencing analysis on lymphocytes obtained from a patient with gastrointestinal neoplasia identified a homozygous intronic *BUBR1* mutation, which creates a de novo splice site that is preferred over the authentic site. Mutant mRNA was targeted by nonsense-mediated mRNA decay, so that no mutant protein was produced. As a consequence, total BUBR1 proteins levels in the patient were significantly reduced compared to controls [170]. *BUBR1* mutations are also found in the hereditary cancer syndrome MVA, which will be discussed below [171,172].

Misregulated expression of BUBR1 has been detected in various tumor types [173]. For instance, reduced BUBR1 protein expression was observed in a subset of thyroid cancer cell lines and colorectal carcinomas [174,175], while increased BUBR1 protein expression was found in breast, gastric, lung, bladder, ovarian, and kidney cancers [176-181].

BubR1 insufficiency in mice did not result in enhanced formation of spontaneous tumors [90]. Nonetheless, challenge with the carcinogen azoxymethane (AOM) in haplo-insufficient BubR1 mice induced rapid and increased formation of lung and intestinal carcinomas compared to wild-type littermates [161]. Treatment of BubR1 hypomorphic mice with the carcinogen 2,4-dimethoxybenzaldehyde (DMBA) resulted in a higher tumor incidence and increased development of lung tumors in comparison with wild-type mice, indicating that *BubR1*$^{H/H}$ mice are more susceptible to DMBA-induced tumorigenesis [91]. A recent study showed that loss of BubR1 acetylation in mice causes a defective spindle assembly checkpoint and promotes the development of spontaneous tumors, including B cell lymphoma, hepatocellular carcinoma and sarcomas [182].

BubR1 and aging

Unexpectedly, mutant mice that express low levels of BubR1 (*BubR1*$^{H/H}$) have a fivefold reduced lifespan and develop multiple progeroid phenotypes and aging-associated phenotypes at an early age [75,90]. BubR1 hypomorphic mice are born without an overt phenotype, but then started to develop cachexia and lordokyphosis (abnormal curvature of the spine). The appearance of lordokyphosis corresponded with atrophy of the gastrocnemius and paraspinal muscle fibers, indicating that BubR1 insufficiency causes severe sarcopenia [75,90]. Dual-energy X-ray absorptiometry (DEXA) measurements revealed that total body fat declined prematurely in *BubR1*$^{H/H}$ mice. Histological analysis of skin confirmed accelerated fat loss, as the thickness of the subcutaneous adipose tissue layer in BubR1 hypomorphic mice was significantly thinner compared to controls [90]. Starting at 2 months of age, *BubR1*$^{H/H}$ mice developed progressive bilateral cataracts that shared features with age-related human cataracts, while no cataracts were observed in wild-type littermates [90]. Other aging-associated phenotypes in BubR1 hypomorphic mice include growth retardation (dwarfism),

facial dysmorphisms, impaired wound healing, infertility, gliosis, arterial wall stiffening and cardiac arrhythmias [75,90,183,184].

Skeletal muscle, fat and eye, tissues that develop early aging-associated phenotypes due to BubR1 insufficiency, have high levels of the senescence markers $p16^{Ink4a}$ and $p19^{Arf}$. Skeletal muscle of BubR1 hypomorphic mice also expressed high levels of other senescence-associated genes, including *Igfbp2*, *Mmp13* and *PAI-1*. In addition, inguinal adipose tissue of $BubR1^{H/H}$ mice stained highly positive for SA-β-galactosidase and *in vivo* 5-bromo-2-deoxyuridine (BrdU) labeling in $BubR1^{H/H}$ mice demonstrated reduced numbers of dividing cells in abdominal muscle and adipose tissue compared to wild-type mice, which are both hallmarks of senescence. Altogether, this indicates that skeletal muscle, fat and eye have high levels of *in vivo* senescence in $BubR1^{H/H}$ mice [75].

Genetic inactivation of $p16^{Ink4a}$ in $BubR1^{H/H}$ mice significantly delayed the onset of lordokyphosis, which was accompanied with reduced muscle atrophy and degeneration. Furthermore, $p16^{Ink4a}$ loss in $BubR1^{H/H}$ mice caused a modest delay in the latency of cataract formation and significantly reduced loss of subcutaneous adipose tissue compared to $BubR1^{H/H}$ mice. However, several other progeroid phenotypes observed in $BubR1^{H/H}$ mice were not improved upon $p16^{Ink4a}$ inactivation, including dwarfism, arterial wall stiffening and infertility [75]. The selective correction by $p16^{Ink4a}$ disruption on specific progeroid phenotypes seems to be dependent on the engagement of $p16^{Ink4a}$ and *in vivo* senescence in $BubR1^{H/H}$ mice. This was supported with the observation that $BubR1^{H/H}$;$p16^{Ink4a-/-}$ mice have significantly reduced levels of senescence in skeletal muscle, fat and eye compared to $BubR1^{H/H}$ mice. Altogether, $p16^{Ink4a}$ inactivation diminished both senescence and aging phenotypes in these tissues, suggesting that BubR1 insufficiency accelerates age-associated phenotypes via $p16^{Ink4a}$-induced senescence (Figure 10) [75].

In contrast, $p19^{Arf}$ and *p53* disruption in $BubR1^{H/H}$ mice accelerated the development of lordokyphosis, muscle wasting, cataract formation, and fat loss. This was accompanied with increased senescence in these tissues, implying that $p19^{Arf}$ and *p53* induction in $BubR1^{H/H}$ mice acts to prevent or suspend senescence, which further support that *in vivo* senescence accelerates aging (Figure 10) [75,185]. Similarly, genetic inactivation of *p21* in $BubR1^{H/H}$ mice mimicked increased senescence levels in muscle and fat and age-related phenotypes in these tissues. Conversely, *p21* disruption delayed cataract formation in $BubR1^{H/H}$ mice, which correlated with reduced senescence in the eye, indicating that p21 drives cataractogenesis in BubR1 progeroid mice (Figure 10) [185].

Furthermore, the notion that BubR1 protein levels decline during natural aging together with the observations that low levels of BubR1 can accelerate aging and several age-related phenotypes, suggests that BubR1 might be a key determinant for life- and healthspan [75,90].

Mosaic variegated aneuploidy

Mosaic variegated aneuploidy (MVA) syndrome is a rare human recessive autosomal disorder characterized by high levels of chromosome missegregation resulting in mosaic aneuploidies, mainly monosomies and trisomies, involving multiple different chromosomes and tissues [171,172]. MVA is a pediatric syndrome with clinical heterogeneic features that include short lifespan, growth deficiency, mental retardation, microcephaly, facial dysmorphisms, cataracts and other eye abnormalities. Children with the MVA syndrome have an increased risk for childhood cancers such as rhabdomyosarcoma, Wilms' tumor and leukemia [171,172,186,187].

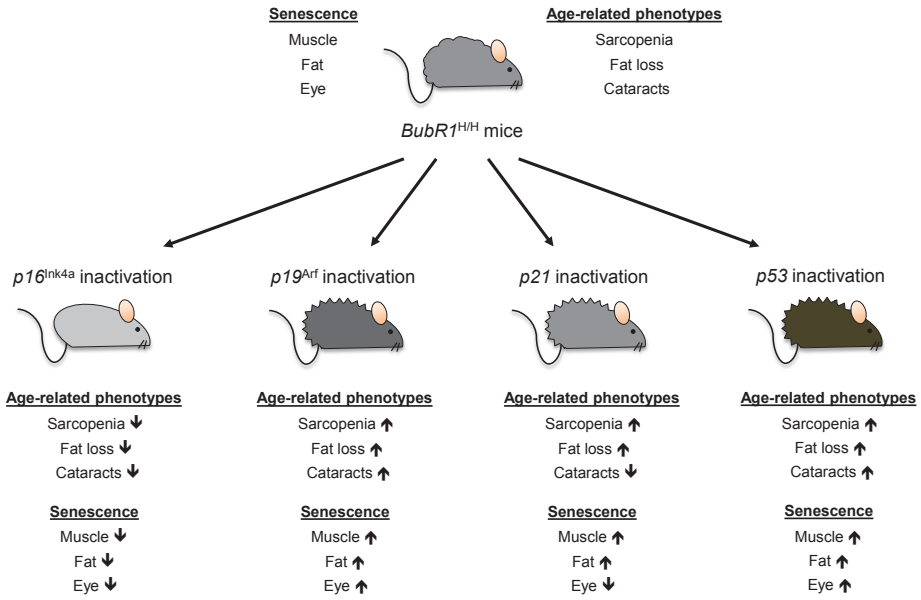

Figure 10. Senescence and age-related phenotypes in the various BubR1 progeroid mouse models. Overview of the gene-inactivation studies in *BubR1*$^{H/H}$ mice, indicating that senescence levels in tissues correlates with the severity of the age-related phenotypes (see text).

The majority of MVA patients have bi-allelic mutations in *BUBR1*, which can be either a missense mutation often located in the kinase domain, a nonsense mutation that results in a premature truncation of the BUBR1 protein or an absent transcript, and/or a single nucleotide variant in an intergenic region 44 kb upstream of a *BUBR1* transcription start site [171,172,187,188].

BUBR1 protein levels are usually very low in MVA patients with *BUBR1* mutations, which can be explained by either non-sense mediated mRNA decay or that mutated proteins are very unstable [170,172,189]. Furthermore, fibroblasts derived from MVA patients with reduced BUBR1 protein levels have an impaired mitotic checkpoint and ectopic BUBR1 expression enabled restoration of mitotic checkpoint activity, indicating that BUBR1 dysfunction causes chromosome segregation errors in MVA patients [189].

5. INFLAMMATION

Introduction

"Inflammaging" refers to a low-grade pro-inflammatory status that occurs during aging in mammals [190,191]. This is reflected by increased expression of genes involved in inflammation and immune response in aged rodents and humans [192-194], and higher levels of inflammatory cytokines in plasma or serum of older humans, like interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα) [195,196]. Others factors that contribute to the pro-inflammatory milieu are the increasing dysfunctional immune system resulting in impaired clearance of pathogens and dysfunctional cells, increased secretion of SASP components by

senescent cells, enhanced activation of the NF-κB pathway, and the reduced efficiency of the autophagy response [5,191,197,198].

Chronic low-grade inflammation is involved in the initiation, propagation, and development of age-related disorders, including obesity and type 2 diabetes [12,199]. Similarly, impaired resolution of inflammation in response to subendothelial lipoproteins is a key event in the pathogenesis of atherosclerosis [200]. Furthermore, age-associated inflammation has been implicated in inhibition of stem cell function, which is another hallmark of aging [5,201]. Finally, persistent inflammation most likely perturbs efficient function of the adaptive immune response that is also observed with aging (immunosenescence) [202]. Altogether, these studies demonstrate that inflammation is associated with and likely contributes to aging and age-related disorders.

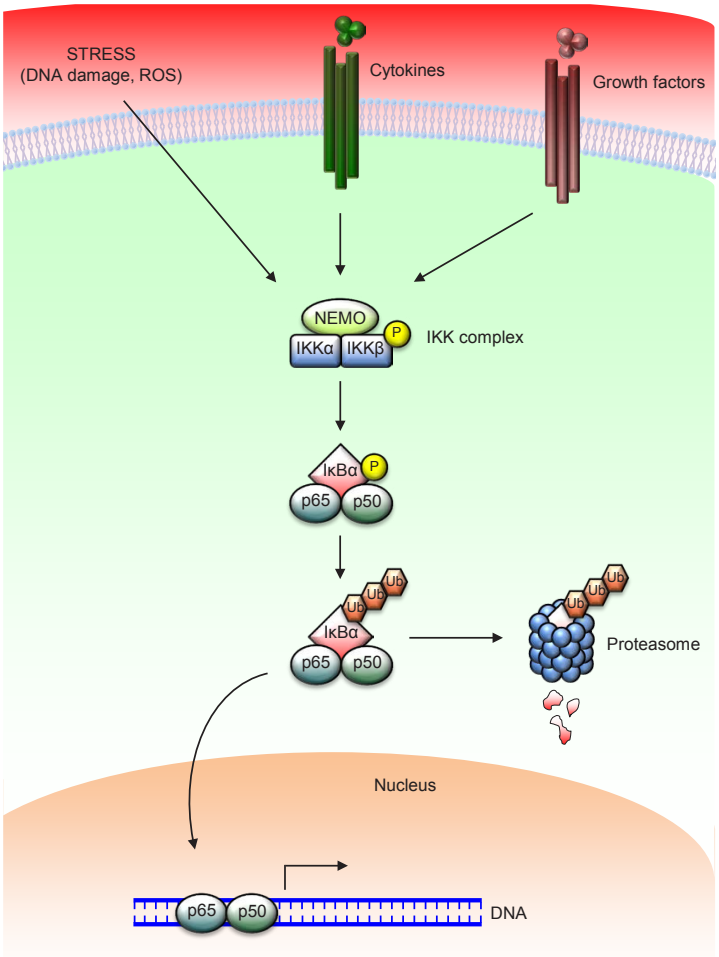

Figure 11. Activation of the canonical NF-κB pathway. Under normal circumstances, the NF-κB complex is inactive and mainly sequestered in the cytoplasm by the inhibitory IκB proteins. Induction of the NF-κB pathway can occur through various stimuli, including pro-inflammatory cytokines, growth factors and DNA damage that result in IKK complex activation. Activated IKK phosphorylates the inhibitor IκBα, leading to its polyubiquitination and subsequent proteasomal degradation. Dissociated NF-κB (heterodimer of p65/p50) can now translocate to the nucleus, bind to κB-response elements and activate transcription of numerous genes, implicated in inflammation, apoptosis and proliferation.

Nuclear factor kappaB (NF-κB)

The transcription factor NF-κB is key component of the cellular response to inflammation, damage, and stress. The NF-κB family consists of five members, p65 (RelA), p50, p52, c-Rel and RelB, which are encoded by *RELA*, *NFKB1*, *NFKB2*, *REL*, and *RELB* [203]. These transcription factors all share a N-terminal Rel homology domain (RHD), which is responsible for homo- and heterodimerization and binding to target sequences termed κB-sites to control gene expression. p65, RelB, and c-Rel also comprise a C-terminal transactivation domain, which enables them to activate gene expression. In order to activate transcription, NF-κB binds to the DNA as a dimer, with the most common one being the heterodimer p65/p50 (canonical pathway) [203,204].

The NF-κB complex is in an inactive state mainly sequestered in the cytoplasm by the inhibitory IκB proteins [204]. NF-κB activation via the canonical pathway is facilitated by the upstream IκB kinase (IKK), which consists of two catalytically activate kinases, IKKα and IKKβ, and a regulator subunit known as NF-κB essential modulator (NEMO) [204]. Numerous stimuli, including pro-inflammatory cytokines, pathogens and growth factors can activate IKK, which in turn phosphorylates IκBα, resulting in its polyubiquitination and subsequent proteasomal degradation (Figure 11) [203,205]. Dissociated NF-κB can now translocate to the nucleus, bind to cognate κB-sequences and activate the transcription of numerous genes, involved in immunity, inflammation, and apoptosis [205].

NF-κB and aging

Various studies have now demonstrated an association with increased NF-κB activity during aging and age-related diseases [206]. Fibroblasts derived from aged donors showed enhanced NF-κB DNA binding activity and increased cell autonomous expression of pro-inflammatory genes [207,208]. Motif module mapping indicated that NF-κB activity was strongly induced in cells from Hutchinson Gilford progeria patients [208]. Moreover, nuclear DNA binding activity of NF-κB is increased in various tissues of aged rodents, including heart, liver, kidney, and brain [209-211]. In addition, chronic activation of NF-κB was observed in multiple age-related disorders, such as muscle wasting, Parkinson's disease, atherosclerosis, and cancer [206,212-215].

The first causal relationship between increased NF-κB and aging was provided in a study where they used inducible genetic blockade of NF-κB in the skin to reveal that continuous NF-κB activity is necessary to inflict aging phenotypes in the skin [208]. Deficiency of mammalian sirtuin-6 (SIRT6) leads to a shortened lifespan and an aging-like phenotype in mice [216]. They showed that the underlying mechanism for accelerated aging is through hyperacetylation of histone H3 lysine 9 (H3K9) at specific promoters resulting in increased p65 promoter occupancy and induced NF-κB-mediated modulation of gene expression, apoptosis and cellular senescence [217]. Indeed, haplo-insufficiency of *p65* was able to attenuate the short lifespan and aging-related phenotypes in *SIRT6*$^{-/-}$ knockout mice [217]. Similarly, a mouse model of XFE progeroid syndrome, a disease of accelerated aging caused by mutations in the xeroderma pigmentosum group F-excision repair cross-complementing rodent repair deficiency complementation group 1 (XPF-ERCC1) DNA repair endonuclease, showed accelerated aging in numerous organs driven by the inability to repair DNA damage [218]. NF-κB activation was increased in tissues of aged wild-type and progeroid, DNA repair-deficient mice, suggesting that accumulation DNA damage is sufficient to activate NF-κB [219]. Genetic depletion of one allele of NF-κB subunit p65 or pharmacological inhibition of the NF-κB-activating kinase, IKK, delayed the onset of

age-related symptoms and pathologies in this progeroid mouse model [219]. Altogether, these studies indicate that chronic activation of NF-κB drives aging and age-related diseases and that genetic and/or pharmacological NF-κB inhibition are at least in part sufficient to attenuate the age-related phenotypes.

Post-translational modifications of RelA/p65

The diversity of the cellular and biological functions of the NF-κB pathway, together with the notion that aberrant activation of NF-κB contributes to aging and age-related diseases, demonstrate the necessity of specific and accurate regulation of its activity [220,221]. Although the activation of the canonical NF-κB pathway is well studied, much less is known about the regulation of nuclear NF-κB activity. Emerging studies indicate that NF-κB undergoes various post-translational modifications, and that these alterations play a key role in regulating the intensity and duration of nuclear NF-κB activity in addition to determining exact transcriptional output [221]. These post-translational modifications can occur at multiple sites of p65, including phosphorylation, acetylation, methylation and ubiquitination [220,221]. A number of phosphorylation sites of p65 have been identified, with most of them located in the N-terminal RHD and the C-terminal transcriptional activation domains (Figure 12). Phosphorylation of these sites results in either increased or decreased transcription levels depending on the stimuli, the phosphorylation sites and the target genes [221].

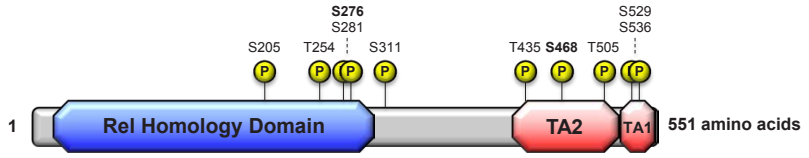

Figure 12. Schematic representation of RelA/p65 with phosphorylation sites. Overview of the p65 protein with its functional domains as indicated: N-terminal Rel Homology domain, responsible for homo- and heterodimerization and binding to target sequences (κB-sites); two C-terminal transactivation domains (TADs), to enable activation of gene expression. All known phosphorylation sites of p65 are indicated (yellow).

One of the best-studied phosphorylation site of p65 is serine 276, which can be phosphorylated by the catalytic subunit of protein kinase A (PKAc) and mitogen- and stress-activated kinase-1 (MSK1) upon activation by upstream lipopolysaccharide (LPS) or TNFα [221-224]. Phosphorylation of serine 276 in response to a variety of stimuli causes enhanced transcriptional activity of NF-κB, most likely through a conformational change that results in either increased or decreased binding of co-factors [221,225,226]. MEFs derived from a knock-in mouse expressing a p65 mutant bearing an alanine instead of a serine at position 276 (S276A) demonstrate a significant drop in NF-κB-mediated transcription [227]. Furthermore, the S276A embryos displayed embryonic lethality with variegated developmental abnormalities. These phenotypes are caused by epigenetic repression as a result from the recruitment of histone deacetylases by the non-phosphorylatable form of NF-κB into the vicinity of genes positioned near NF-κB binding sites [227]. In contrast, knock-in mice where serine 276 was replaced by an aspartic acid (S276D) displayed a progressive, systemic hyperinflammatory condition leading to severe runting and death between postnatal days 8-20 [228]. This “phospho-mimetic” mutation caused constitutive NF-κB activation that triggers systemic inflammation through increased TNF production [228].

Another phosphorylation site of p65, serine 468 has been implicated in termination of NF-κB-mediated transcription [229,230]. Phosphorylation of serine 468 is mediated by various kinases, including glycogen synthase kinase 3 beta (GSK-3β), IKKβ, and IKKε [231-233]. Two independent groups demonstrated that TNFα-induced phosphorylation of p65 at serine 468 allows the binding of copper metabolism (Murr1) containing domain 1 (Commd1) and cullin 2, components of a multimeric ubiquitination ligase complex mediating ubiquitination and subsequent proteasomal degradation of chromatin bound p65 [229,230]. In addition, proteasomal p65 elimination was restricted to a subset of NF-κB target genes, indicating that phosphorylation of serine 468 contributes to the selective termination of NF-κB-dependent gene expression [230]. NF-κB target gene expression analysis after reconstitution of NF-κB p65-deficient MEFs with the wild-type protein or phosphorylation-defect mutants confirmed the highly target gene-specific transcription for this phosphorylation site [234]. However, mouse studies that assess the importance of phosphorylation at p65 serine 468 are still lacking.

6. THESIS AIM AND OUTLINE

The number of people reaching old age is expected to increase dramatically, and concomitantly age-related diseases, such as arthritis, diabetes, neurodegenerative disorders, heart disease and cancer. To improve health and quality of life of the elderly, it will be necessary to identify and understand the molecular pathways and events that drive aging and aging-associated diseases. The central aim of this thesis was to elucidate the contribution of cellular senescence and inflammation in aging and age-related diseases using a number of newly generated genetically modified mouse models. Our study revealed that cellular senescence drives several age-related phenotypes and that removal of senescent cells can delay tissue dysfunction and extend healthy lifespan.

In chapter 2, we examined whether $p16^{Ink4a}$-positive senescent cells are causally implicated in age-related dysfunction and whether their removal has beneficial effects. Using a novel transgene, termed *INK-ATTAC*, that enables the inducible elimination of $p16^{Ink4a}$-positive senescent cells upon administration of a drug, we found that life-long removal of senescent cells in a BubR1 progeroid mouse model selectively delayed the onset of $p16^{Ink4a}$-dependent age-related pathologies. Furthermore, late-life clearance of senescent cells delayed progression of already established age-related phenotypes.

Mutations that cause a reduction in BubR1 levels cause aneuploidy, shorten lifespan, and accelerate the onset of aging-associated disorders in mice. In addition, reduced BubR1 expression is a characteristic of chronological aging. To investigate whether overexpression of BubR1 can extend healthy lifespan, we generated transgenic mice expressing high amounts of BubR1 (chapter 3). We discovered that sustained overexpression of BubR1 extends lifespan, reduced senescence and neoplastic transformation, and delayed age-related deterioration in several tissues. These tissues were protected against genome instability through improved correction of mitotic checkpoint and/or kinetochore-microtubule attachment defects.

In chapter 4, we assessed the biological impact of a mono-allelic *BubR1* MVA mutation, that is found in an MVA patient with bi-allelic *BUBR1* mutations. We demonstrated that mice heterozygous for a *BubR1* MVA mutation have a reduced median and maximum lifespan and develop several aging-associated phenotypes at an accelerated rate. Moreover, we identified that accelerated deterioration of skeletal muscle and age-related fat loss was associated with increased levels of the senescence markers $p16^{Ink4a}$ and $p19^{Arf}$ in these tissues.

Inflammation, including the pro-inflammatory phenotype induced by the SASP of senescent cells, is thought to play an important role in aging and age-related disease. Studies conducted to better understand the molecular mechanism of inflammation are described in chapter 5. Specifically, we examined the *in vivo* importance of the phosphorylation site NF-κB p65 at residue 467 (corresponding to human p65 serine 468), using two newly generated knock-in mouse models in which we substituted serine 467 with either an alanine or an aspartic acid, resulting in $p65^{S467A}$ ("non-phosphorylatable") or $p65^{S467D}$ ("phospho-mimetic") mice, respectively. MEFs derived from these $p65^{S467A}$ mice have reduced total p65 protein levels, showed impaired activation of NF-κB-mediated gene expression, and decreased cell viability. Furthermore, *in vivo* TNF-induced NF-κB activation revealed diminished expression of inflammatory genes in various tissues of $p65^{S467A}$ mice, while expression of anti-apoptotic genes was unaffected.

Chapter 6 contains the main conclusions of this thesis as well as a general discussion and future outlook.

REFERENCES

1. Wang H, Dwyer-Lindgren L, Lofgren KT, Rajaratnam JK, Marcus JR, et al. (2012) Age-specific and sex-specific mortality in 187 countries, 1970-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380: 2071–2094. doi:10.1016/S0140-6736(12)61719-X.
2. Newgard CB, Sharpless NE (2013) Coming of age: molecular drivers of aging and therapeutic opportunities. J Clin Invest 123: 946–950. doi:10.1172/JCI68833.
3. Campisi J (2013) Aging, cellular senescence, and cancer. Annu Rev Physiol 75: 685–705. doi:10.1146/annurev-physiol-030212-183653.
4. Niccoli T, Partridge L (2012) Ageing as a risk factor for disease. Curr Biol 22: R741–R752. doi:10.1016/j.cub.2012.07.024.
5. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153: 1194–1217. doi:10.1016/j.cell.2013.05.039.
6. Ito K, Barnes PJ (2009) COPD as a disease of accelerated lung aging. Chest 135: 173–180. doi:10.1378/chest.08-1419.
7. Price JS, Waters JG, Darrah C, Pennington C, Edwards DR, et al. (2002) The role of chondrocyte senescence in osteoarthritis. Aging Cell 1: 57–65.
8. DePinho RA (2000) The age of cancer. Nature 408: 248–254. doi:10.1038/35041694.
9. Kenyon CJ (2010) The genetics of ageing. Nature 464: 504–512. doi:10.1038/nature08980.
10. Gems D, Partridge L (2013) Genetics of longevity in model organisms: debates and paradigm shifts. Annu Rev Physiol 75: 621–644. doi:10.1146/annurev-physiol-030212-183712.
11. Fontana L, Partridge L, Longo VD (2010) Extending healthy life span--from yeast to humans. Science 328: 321–326. doi:10.1126/science.1172539.
12. Barzilai N, Huffman DM, Muzumdar RH, Bartke A (2012) The critical role of metabolic pathways in aging. Diabetes 61: 1315–1322. doi:10.2337/db11-1300.
13. Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A C. elegans mutant that lives twice as long as wild type. Nature 366: 461–464. doi:10.1038/366461a0.
14. Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, Popovich IG, et al. (2008) Metformin slows down aging and extends life span of female SHR mice. Cell Cycle 7: 2769–2773.
15. Mair W, Morantte I, Rodrigues APC, Manning G, Montminy M, et al. (2011) Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB. Nature 470: 404–408. doi:10.1038/nature09706.
16. Martin-Montalvo A, Mercken EM, Mitchell SJ, Palacios HH, Mote PL, et al. (2013) Metformin improves healthspan and lifespan in mice. Nat Commun 4: 2192. doi:10.1038/ncomms3192.
17. Selman C, Tullet JMA, Wieser D, Irvine E, Lingard SJ, et al. (2009) Ribosomal protein S6 kinase 1 signaling regulates mammalian life span. Science 326: 140–144. doi:10.1126/science.1177221.
18. Johnson SC, Rabinovitch PS, Kaeberlein M (2013) mTOR is a key modulator of ageing and age-related disease. Nature 493: 338–345. doi:10.1038/nature11861.
19. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, et al. (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460: 392–395. doi:10.1038/nature08221.

20. Birse RT, Choi J, Reardon K, Rodriguez J, Graham S, et al. (2010) High-fat-diet-induced obesity and heart dysfunction are regulated by the TOR pathway in Drosophila. Cell Metabolism 12: 533–544. doi:10.1016/j.cmet.2010.09.014.
21. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, et al. (2004) Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature 431: 200–205. doi:10.1038/nature02866.
22. Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, et al. (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 325: 201–204. doi:10.1126/science.1173635.
23. Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, et al. (2012) Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. Nature 489: 318–321. doi:10.1038/nature11432.
24. Colman RJ, Beasley TM, Kemnitz JW, Johnson SC, Weindruch R, et al. (2014) Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. Nat Commun 5: 3557. doi:10.1038/ncomms4557.
25. Hoeijmakers JHJ (2009) DNA damage, aging, and cancer. N Engl J Med 361: 1475–1485. doi:10.1056/NEJMra0804615.
26. Lord CJ, Ashworth A (2012) The DNA damage response and cancer therapy. Nature 481: 287–294. doi:10.1038/nature10760.
27. Faggioli F, Wang T, Vijg J, Montagna C (2012) Chromosome-specific accumulation of aneuploidy in the aging mouse brain. Hum Mol Genet 21: 5246–5253. doi:10.1093/hmg/dds375.
28. Weaver BAA, Silk AD, Montagna C, Verdier-Pinard P, Cleveland DW (2007) Aneuploidy acts both oncogenically and as a tumor suppressor. Cancer Cell 11: 25–36. doi:10.1016/j.ccr.2006.12.003.
29. Lushnikova T, Bouska A, Odvody J, Dupont WD, Eischen CM (2011) Aging mice have increased chromosome instability that is exacerbated by elevated Mdm2 expression. Oncogene 30: 4622–4631. doi:10.1038/onc.2011.172.
30. Iourov IY, Vorsanova SG, Liehr T, Yurov YB (2009) Aneuploidy in the normal, Alzheimer's disease and ataxia-telangiectasia brain: differential expression and pathological meaning. Neurobiol Dis 34: 212–220. doi:10.1016/j.nbd.2009.01.003.
31. Jacobs KB, Yeager M, Zhou W, Wacholder S, Wang Z, et al. (2012) Detectable clonal mosaicism and its relationship to aging and cancer. Nat Genet 44: 651–658. doi:10.1038/ng.2270.
32. Laurie CC, Laurie CA, Rice K, Doheny KF, Zelnick LR, et al. (2012) Detectable clonal mosaicism from birth to old age and its relationship to cancer. Nat Genet 44: 642–650. doi:10.1038/ng.2271.
33. Talens RP, Christensen K, Putter H, Willemsen G, Christiansen L, et al. (2012) Epigenetic variation during the adult lifespan: cross-sectional and longitudinal data on monozygotic twin pairs. Aging Cell 11: 694–703. doi:10.1111/j.1474-9726.2012.00835.x.
34. Greer EL, Maures TJ, Hauswirth AG, Green EM, Leeman DS, et al. (2010) Members of the H3K4 trimethylation complex regulate lifespan in a germline-dependent manner in C. elegans. Nature 466: 383–387. doi:10.1038/nature09195.
35. Houtkooper RH, Williams RW, Auwerx J (2010) Metabolic networks of longevity. Cell 142: 9–14. doi:10.1016/j.cell.2010.06.029.
36. Kanfi Y, Naiman S, Amir G, Peshti V, Zinman G, et al. (2012) The sirtuin SIRT6 regulates lifespan in male mice. Nature 483: 218–221. doi:10.1038/nature10815.

37. Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE (2009) Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem 78: 959–991. doi:10.1146/annurev.biochem.052308.114844.
38. Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. Nature 475: 324–332. doi:10.1038/nature10317.
39. Koga H, Kaushik S, Cuervo AM (2011) Protein homeostasis and aging: The importance of exquisite quality control. Ageing Res Rev 10: 205–215. doi:10.1016/j.arr.2010.02.001.
40. Mizushima N, Levine B, Cuervo AM, Klionsky DJ (2008) Autophagy fights disease through cellular self-digestion. Nature 451: 1069–1075. doi:10.1038/nature06639.
41. Min J-N, Whaley RA, Sharpless NE, Lockyer P, Portbury AL, et al. (2008) CHIP deficiency decreases longevity, with accelerated aging phenotypes accompanied by altered protein quality control. Mol Cell Biol 28: 4018–4025. doi:10.1128/MCB.00296-08.
42. Rubinsztein DC, Mariño G, Kroemer G (2011) Autophagy and aging. Cell 146: 682–695. doi:10.1016/j.cell.2011.07.030.
43. Tomaru U, Takahashi S, Ishizu A, Miyatake Y, Gohda A, et al. (2012) Decreased proteasomal activity causes age-related phenotypes and promotes the development of metabolic abnormalities. Am J Pathol 180: 963–972. doi:10.1016/j.ajpath.2011.11.012.
44. Eisenberg T, Knauer H, Schauer A, Büttner S, Ruckenstuhl C, et al. (2009) Induction of autophagy by spermidine promotes longevity. Nat Cell Biol 11: 1305–1314. doi:10.1038/ncb1975.
45. Liu G, Rogers J, Murphy CT, Rongo C (2011) EGF signalling activates the ubiquitin proteasome system to modulate C. elegans lifespan. EMBO J 30: 2990–3003. doi:10.1038/emboj.2011.195.
46. Kruegel U, Robison B, Dange T, Kahlert G, Delaney JR, et al. (2011) Elevated proteasome capacity extends replicative lifespan in Saccharomyces cerevisiae. PLoS Genet 7: e1002253. doi:10.1371/journal.pgen.1002253.
47. Harman D (1956) Aging: a theory based on free radical and radiation chemistry. J Gerontol 11: 298–300.
48. Doonan R, McElwee JJ, Matthijssens F, Walker GA, Houthoofd K, et al. (2008) Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in Caenorhabditis elegans. Genes & Development 22: 3236–3241. doi:10.1101/gad.504808.
49. Van Raamsdonk JM, Hekimi S (2009) Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in Caenorhabditis elegans. PLoS Genet 5: e1000361. doi:10.1371/journal.pgen.1000361.
50. Zhang Y, Ikeno Y, Qi W, Chaudhuri A, Li Y, et al. (2009) Mice deficient in both Mn superoxide dismutase and glutathione peroxidase-1 have increased oxidative damage and a greater incidence of pathology but no reduction in longevity. J Gerontol A Biol Sci Med Sci 64: 1212–1220. doi:10.1093/gerona/glp132.
51. Pérez VI, Van Remmen H, Bokov A, Epstein CJ, Vijg J, et al. (2009) The overexpression of major antioxidant enzymes does not extend the lifespan of mice. Aging Cell 8: 73–75. doi:10.1111/j.1474-9726.2008.00449.x.
52. Edgar D, Shabalina I, Camara Y, Wredenberg A, Calvaruso MA, et al. (2009) Random point mutations with major effects on protein-coding genes are the driving force behind premature aging in mtDNA mutator mice. Cell Metabolism 10: 131–138. doi:10.1016/j.cmet.2009.06.010.

53. Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, et al. (2005) Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. Science 309: 481–484. doi:10.1126/science.1112125.
54. Green DR, Galluzzi L, Kroemer G (2011) Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. Science 333: 1109–1112. doi:10.1126/science.1201940.
55. Hiona A, Sanz A, Kujoth GC, Pamplona R, Seo AY, et al. (2010) Mitochondrial DNA mutations induce mitochondrial dysfunction, apoptosis and sarcopenia in skeletal muscle of mitochondrial DNA mutator mice. PLoS ONE 5: e11468. doi:10.1371/journal.pone.0011468.
56. Sharpless NE, DePinho RA (2007) How stem cells age and why this makes us grow old. Nat Rev Mol Cell Biol 8: 703–713. doi:10.1038/nrm2241.
57. Rossi DJ, Bryder D, Seita J, Nussenzweig A, Hoeijmakers J, et al. (2007) Deficiencies in DNA damage repair limit the function of haematopoietic stem cells with age. Nature 447: 725–729. doi:10.1038/nature05862.
58. Conboy IM, Rando TA (2005) Aging, stem cells and tissue regeneration: lessons from muscle. Cell Cycle 4: 407–410.
59. Anversa P, Rota M, Urbanek K, Hosoda T, Sonnenblick EH, et al. (2005) Myocardial aging--a stem cell problem. Basic Res Cardiol 100: 482–493. doi:10.1007/s00395-005-0554-3.
60. Flores I, Cayuela ML, Blasco MA (2005) Effects of telomerase and telomere length on epidermal stem cell behavior. Science 309: 1253–1256. doi:10.1126/science.1115025.
61. Chakkalakal JV, Jones KM, Basson MA, Brack AS (2012) The aged niche disrupts muscle stem cell quiescence. Nature 490: 355–360. doi:10.1038/nature11438.
62. Lavasani M, Robinson AR, Lu A, Song M, Feduska JM, et al. (2012) Muscle-derived stem/progenitor cell dysfunction limits healthspan and lifespan in a murine progeria model. Nat Commun 3: 608. doi:10.1038/ncomms1611.
63. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, et al. (2005) Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 433: 760–764. doi:10.1038/nature03260.
64. Kuilman T, Michaloglou C, Mooi WJ, Peeper DS (2010) The essence of senescence. Genes & Development 24: 2463–2479. doi:10.1101/gad.1971610.
65. van Deursen JM (2014) The role of senescent cells in ageing. 509: 439–446. doi:10.1038/nature13193.
66. Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. Exp Cell Res 25: 585–621.
67. Shay JW, Wright WE (2000) Hayflick, his limit, and cellular ageing. Nat Rev Mol Cell Biol 1: 72-76. doi:10.1038/35036093.
68. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, et al. (1998) Extension of life-span by introduction of telomerase into normal human cells. Science 279: 349–352.
69. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 88: 593–602.
70. Tchkonia T, Zhu Y, van Deursen J, Campisi J, Kirkland JL (2013) Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J Clin Invest 123: 966–972. doi:10.1172/JCI64098.
71. Storer M, Mas A, Robert-Moreno A, Pecoraro M, Ortells MC, et al. (2013) Senescence is a developmental mechanism that contributes to embryonic growth and patterning. Cell 155: 1119–1130. doi:10.1016/j.cell.2013.10.041.

72. Muñoz-Espín D, Cañamero M, Maraver A, Gómez-López G, Contreras J, et al. (2013) Programmed cell senescence during mammalian embryonic development. Cell 155: 1104–1118. doi:10.1016/j.cell.2013.10.019.
73. Krizhanovsky V, Yon M, Dickins RA, Hearn S, Simon J, et al. (2008) Senescence of activated stellate cells limits liver fibrosis. Cell 134: 657–667. doi:10.1016/j.cell.2008.06.049.
74. Jun J-I, Lau LF (2010) The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. Nat Cell Biol 12: 676–685. doi:10.1038/ncb2070.
75. Baker DJ, Perez-Terzic C, Jin F, Pitel KS, Niederländer NJ, et al. (2008) Opposing roles for p16Ink4a and p19Arf in senescence and ageing caused by BubR1 insufficiency. Nat Cell Biol 10: 825–836. doi:10.1038/ncb1744.
76. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, et al. (2003) A DNA damage checkpoint response in telomere-initiated senescence. Nature 426: 194–198. doi:10.1038/nature02118.
77. Takai H, Smogorzewska A, de Lange T (2003) DNA damage foci at dysfunctional telomeres. Curr Biol 13: 1549–1556.
78. Fumagalli M, Rossiello F, Clerici M, Barozzi S, Cittaro D, et al. (2012) Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. Nat Cell Biol 14: 355–365. doi:10.1038/ncb2466.
79. Zglinicki von T, Saretzki G, Ladhoff J, d'Adda di Fagagna F, Jackson SP (2005) Human cell senescence as a DNA damage response. Mech Ageing Dev 126: 111–117. doi:10.1016/j.mad.2004.09.034.
80. Sedelnikova OA, Horikawa I, Zimonjic DB, Popescu NC, Bonner WM, et al. (2004) Senescing human cells and ageing mice accumulate DNA lesions with unrepairable double-strand breaks. Nat Cell Biol 6: 168–170. doi:10.1038/ncb1095.
81. Wang C, Jurk D, Maddick M, Nelson G, Martin-Ruiz C, et al. (2009) DNA damage response and cellular senescence in tissues of aging mice. Aging Cell 8: 311–323. doi:10.1111/j.1474-9726.2009.00481.x.
82. Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, et al. (2002) A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. Cell 109: 335–346.
83. Barascu A, Le Chalony C, Pennarun G, Genet D, Imam N, et al. (2012) Oxidative stress induces an ATM-independent senescence pathway through p38 MAPK-mediated lamin B1 accumulation. EMBO J 31: 1080–1094. doi:10.1038/emboj.2011.492.
84. Parrinello S, Samper E, Krtolica A, Goldstein J, Melov S, et al. (2003) Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. Nat Cell Biol 5: 741–747. doi:10.1038/ncb1024.
85. Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, et al. (2006) Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature 444: 638–642. doi:10.1038/nature05327.
86. Guney I, Wu S, Sedivy JM (2006) Reduced c-Myc signaling triggers telomere-independent senescence by regulating Bmi-1 and p16(INK4a). Proc Natl Acad Sci USA 103: 3645–3650. doi:10.1073/pnas.0600069103.
87. Campisi J (2005) Suppressing cancer: the importance of being senescent. Science 309: 886–887. doi:10.1126/science.1116801.
88. Narita M, Nũnez S, Heard E, Narita M, Lin AW, et al. (2003) Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. Cell 113: 703–716.

89. Munro J, Barr NI, Ireland H, Morrison V, Parkinson EK (2004) Histone deacetylase inhibitors induce a senescence-like state in human cells by a p16-dependent mechanism that is independent of a mitotic clock. Exp Cell Res 295: 525–538. doi:10.1016/j.yexcr.2004.01.017.
90. Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, et al. (2004) BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. Nat Genet 36: 744–749. doi:10.1038/ng1382.
91. Baker DJ, Jeganathan KB, Malureanu L, Perez-Terzic C, Terzic A, et al. (2006) Early aging-associated phenotypes in Bub3/Rae1 haploinsufficient mice. J Cell Biol 172: 529–540. doi:10.1083/jcb.200507081.
92. Campisi J, d'Adda di Fagagna F (2007) Cellular senescence: when bad things happen to good cells. 8: 729–740. doi:10.1038/nrm2233.
93. Adams PD (2009) Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. Mol Cell 36: 2–14. doi:10.1016/j.molcel.2009.09.021.
94. Rodier F, Coppé J-P, Patil CK, Hoeijmakers WAM, Muñoz DP, et al. (2009) Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol 11: 973–979. doi:10.1038/ncb1909.
95. Levine AJ, Oren M (2009) The first 30 years of p53: growing ever more complex. Nat Rev Cancer 9: 749–758. doi:10.1038/nrc2723.
96. Freund A, Patil CK, Campisi J (2011) p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. EMBO J 30: 1536–1548. doi:10.1038/emboj.2011.69.
97. Passos JF, Nelson G, Wang C, Richter T, Simillion C, et al. (2010) Feedback between p21 and reactive oxygen production is necessary for cell senescence. Mol Syst Biol 6: 347. doi:10.1038/msb.2010.5.
98. Purvis JE, Karhohs KW, Mock C, Batchelor E, Loewer A, et al. (2012) p53 dynamics control cell fate. Science 336: 1440–1444. doi:10.1126/science.1218351.
99. Ohtani N, Yamakoshi K, Takahashi A, Hara E (2004) The p16INK4a-RB pathway: molecular link between cellular senescence and tumor suppression. J Med Invest 51: 146–153.
100. Collins CJ, Sedivy JM (2003) Involvement of the INK4a/Arf gene locus in senescence. Aging Cell 2: 145–150.
101. Krishnamurthy J, Torrice C, Ramsey MR, Kovalev GI, Al-Regaiey K, et al. (2004) Ink4a/Arf expression is a biomarker of aging. J Clin Invest 114: 1299–1307. doi:10.1172/JCI22475.
102. Liu Y, Sanoff HK, Cho H, Burd CE, Torrice C, et al. (2009) Expression of p16(INK4a) in peripheral blood T-cells is a biomarker of human aging. Aging Cell 8: 439–448. doi:10.1111/j.1474-9726.2009.00489.x.
103. Ressler S, Bartkova J, Niederegger H, Bartek J, Scharffetter-Kochanek K, et al. (2006) p16INK4A is a robust in vivo biomarker of cellular aging in human skin. Aging Cell 5: 379–389. doi:10.1111/j.1474-9726.2006.00231.x.
104. Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM (2006) Cellular senescence in aging primates. Science 311: 1257–1257. doi:10.1126/science.1122446.
105. Dimri GP, Lee X, Basile G, Acosta M, Scott G, et al. (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA 92: 9363–9367.
106. Kurz DJ, Decary S, Hong Y, Erusalimsky JD (2000) Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. J Cell Sci 113 (Pt 20): 3613–3622.

107. Freund A, Laberge R-M, Demaria M, Campisi J (2012) Lamin B1 loss is a senescence-associated biomarker. Mol Biol Cell 23: 2066–2075. doi:10.1091/mbc.E11-10-0884.
108. Shimi T, Butin-Israeli V, Adam SA, Hamanaka RB, Goldman AE, et al. (2011) The role of nuclear lamin B1 in cell proliferation and senescence. Genes & Development 25: 2579–2593. doi:10.1101/gad.179515.111.
109. Shah PP, Donahue G, Otte GL, Capell BC, Nelson DM, et al. (2013) Lamin B1 depletion in senescent cells triggers large-scale changes in gene expression and the chromatin landscape. Genes & Development 27: 1787–1799. doi:10.1101/gad.223834.113.
110. Narita M, Narita M, Krizhanovsky V, Nũnez S, Chicas A, et al. (2006) A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. Cell 126: 503–514. doi:10.1016/j.cell.2006.05.052.
111. Shelton DN, Chang E, Whittier PS, Choi D, Funk WD (1999) Microarray analysis of replicative senescence. Curr Biol 9: 939–945.
112. Zhang H, Pan K-H, Cohen SN (2003) Senescence-specific gene expression fingerprints reveal cell-type-dependent physical clustering of up-regulated chromosomal loci. Proc Natl Acad Sci USA 100: 3251–3256. doi:10.1073/pnas.2627983100.
113. Rodier F, Muñoz DP, Teachenor R, Chu V, Le O, et al. (2011) DNA-SCARS: distinct nuclear structures that sustain damage-induced senescence growth arrest and inflammatory cytokine secretion. J Cell Sci 124: 68–81. doi:10.1242/jcs.071340.
114. Campisi J, Andersen JK, Kapahi P, Melov S (2011) Cellular senescence: a link between cancer and age-related degenerative disease? Semin Cancer Biol 21: 354–359. doi:10.1016/j.semcancer.2011.09.001.
115. Coppé J-P, Patil CK, Rodier F, Sun Y, Muñoz DP, et al. (2008) Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 6: 2853–2868. doi:10.1371/journal.pbio.0060301.
116. Coppé J-P, Rodier F, Patil CK, Freund A, Desprez P-Y, et al. (2011) Tumor suppressor and aging biomarker p16(INK4a) induces cellular senescence without the associated inflammatory secretory phenotype. Journal of Biological Chemistry 286: 36396–36403. doi:10.1074/jbc.M111.257071.
117. Pazolli E, Alspach E, Milczarek A, Prior J, Piwnica-Worms D, et al. (2012) Chromatin remodeling underlies the senescence-associated secretory phenotype of tumor stromal fibroblasts that supports cancer progression. Cancer Res 72: 2251–2261. doi:10.1158/0008-5472.CAN-11-3386.
118. Kuilman T, Michaloglou C, Vredeveld LCW, Douma S, van Doorn R, et al. (2008) Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. Cell 133: 1019–1031. doi:10.1016/j.cell.2008.03.039.
119. Salminen A, Kauppinen A, Kaarniranta K (2012) Emerging role of NF-κB signaling in the induction of senescence-associated secretory phenotype (SASP). Cell Signal 24: 835–845. doi:10.1016/j.cellsig.2011.12.006.
120. Chien Y, Scuoppo C, Wang X, Fang X, Balgley B, et al. (2011) Control of the senescence-associated secretory phenotype by NF-κB promotes senescence and enhances chemosensitivity. Genes & Development 25: 2125–2136. doi:10.1101/gad.17276711.
121. Kaplon J, Zheng L, Meissl K, Chaneton B, Selivanov VA, et al. (2013) A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. Nature 498: 109–112. doi:10.1038/nature12154.

122. Coppé J-P, Kauser K, Campisi J, Beausejour CM (2006) Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. J Biol Chem 281: 29568–29574. doi:10.1074/jbc.M603307200.
123. Laberge R-M, Awad P, Campisi J, Desprez P-Y (2012) Epithelial-mesenchymal transition induced by senescent fibroblasts. Cancer Microenviron 5: 39–44. doi:10.1007/s12307-011-0069-4.
124. Freund A, Orjalo AV, Desprez P-Y, Campisi J (2010) Inflammatory networks during cellular senescence: causes and consequences. Trends Mol Med 16: 238–246. doi:10.1016/j.molmed.2010.03.003.
125. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, et al. (2007) Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science 317: 807–810. doi:10.1126/science.1144090.
126. Binet R, Ythier D, Robles AI, Collado M, Larrieu D, et al. (2009) WNT16B is a new marker of cellular senescence that regulates p53 activity and the phosphoinositide 3-kinase/AKT pathway. Cancer Res 69: 9183–9191. doi:10.1158/0008-5472.CAN-09-1016.
127. Yang G, Rosen DG, Zhang Z, Bast RC, Mills GB, et al. (2006) The chemokine growth-regulated oncogene 1 (Gro-1) links RAS signaling to the senescence of stromal fibroblasts and ovarian tumorigenesis. Proc Natl Acad Sci USA 103: 16472–16477. doi:10.1073/pnas.0605752103.
128. Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J (2001) Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. Proc Natl Acad Sci USA 98: 12072–12077. doi:10.1073/pnas.211053698.
129. Liu D, Hornsby PJ (2007) Senescent human fibroblasts increase the early growth of xenograft tumors via matrix metalloproteinase secretion. Cancer Res 67: 3117–3126. doi:10.1158/0008-5472.CAN-06-3452.
130. Parrinello S, Coppé J-P, Krtolica A, Campisi J (2005) Stromal-epithelial interactions in aging and cancer: senescent fibroblasts alter epithelial cell differentiation. J Cell Sci 118: 485–496. doi:10.1242/jcs.01635.
131. Kang T-W, Yevsa T, Woller N, Hoenicke L, Wuestefeld T, et al. (2011) Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. Nature 479: 547–551. doi:10.1038/nature10599.
132. Lawless C, Wang C, Jurk D, Merz A, Zglinicki TV, et al. (2010) Quantitative assessment of markers for cell senescence. Exp Gerontol 45: 772–778. doi:10.1016/j.exger.2010.01.018.
133. Jeyapalan JC, Ferreira M, Sedivy JM, Herbig U (2007) Accumulation of senescent cells in mitotic tissue of aging primates. Mech Ageing Dev 128: 36–44. doi:10.1016/j.mad.2006.11.008.
134. Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A, et al. (2006) p16INK4a induces an age-dependent decline in islet regenerative potential. Nature 443: 453–457. doi:10.1038/nature05092.
135. Erusalimsky JD, Kurz DJ (2005) Cellular senescence in vivo: its relevance in ageing and cardiovascular disease. Exp Gerontol 40: 634–642. doi:10.1016/j.exger.2005.04.010.
136. Gorenne I, Kavurma M, Scott S, Bennett M (2006) Vascular smooth muscle cell senescence in atherosclerosis. Cardiovasc Res 72: 9–17. doi:10.1016/j.cardiores.2006.06.004.
137. Kassem M, Marie PJ (2011) Senescence-associated intrinsic mechanisms of osteoblast dysfunctions. Aging Cell 10: 191–197. doi:10.1111/j.1474-9726.2011.00669.x.
138. Roberts S, Evans EH, Kletsas D, Jaffray DC, Eisenstein SM (2006) Senescence in human intervertebral discs. Eur Spine J 15 Suppl 3: S312–S316. doi:10.1007/s00586-006-0126-8.

139. Shane Anderson A, Loeser RF (2010) Why is osteoarthritis an age-related disease? Best Pract Res Clin Rheumatol 24: 15–26. doi:10.1016/j.berh.2009.08.006.
140. Bitto A, Sell C, Crowe E, Lorenzini A, Malaguti M, et al. (2010) Stress-induced senescence in human and rodent astrocytes. Exp Cell Res 316: 2961–2968. doi:10.1016/j.yexcr.2010.06.021.
141. Salminen A, Ojala J, Kaarniranta K, Haapasalo A, Hiltunen M, et al. (2011) Astrocytes in the aging brain express characteristics of senescence-associated secretory phenotype. Eur J Neurosci 34: 3–11. doi:10.1111/j.1460-9568.2011.07738.x.
142. Xue W, Zender L, Miething C, Dickins RA, Hernando E, et al. (2007) Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 445: 656–660. doi:10.1038/nature05529.
143. Dorshkind K, Montecino-Rodriguez E, Signer RAJ (2009) The ageing immune system: is it ever too old to become young again? Nat Rev Immunol 9: 57–62. doi:10.1038/nri2471.
144. Wang J, Geiger H, Rudolph KL (2011) Immunoaging induced by hematopoietic stem cell aging. Curr Opin Immunol 23: 532–536. doi:10.1016/j.coi.2011.05.004.
145. Nikolich-Zugich J (2008) Ageing and life-long maintenance of T-cell subsets in the face of latent persistent infections. Nat Rev Immunol 8: 512–522. doi:10.1038/nri2318.
146. Kim WY, Sharpless NE (2006) The regulation of INK4/ARF in cancer and aging. Cell 127: 265–275. doi:10.1016/j.cell.2006.10.003.
147. Collado M, Blasco MA, Serrano M (2007) Cellular senescence in cancer and aging. Cell 130: 223–233. doi:10.1016/j.cell.2007.07.003.
148. Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, et al. (2013) A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat Cell Biol 15: 978–990. doi:10.1038/ncb2784.
149. Nelson G, Wordsworth J, Wang C, Jurk D, Lawless C, et al. (2012) A senescent cell bystander effect: senescence-induced senescence. Aging Cell 11: 345–349. doi:10.1111/j.1474-9726.2012.00795.x.
150. Jablonski SA, Chan GK, Cooke CA, Earnshaw WC, Yen TJ (1998) The hBUB1 and hBUBR1 kinases sequentially assemble onto kinetochores during prophase with hBUBR1 concentrating at the kinetochore plates in mitosis. Chromosoma 107: 386–396.
151. Taylor SS, Ha E, McKeon F (1998) The human homologue of Bub3 is required for kinetochore localization of Bub1 and a Mad3/Bub1-related protein kinase. J Cell Biol 142: 1–11.
152. Lara-Gonzalez P, Scott MIF, Diez M, Sen O, Taylor SS (2011) BubR1 blocks substrate recruitment to the APC/C in a KEN-box-dependent manner. J Cell Sci 124: 4332–4345. doi:10.1242/jcs.094763.
153. D'Arcy S, Davies OR, Blundell TL, Bolanos-Garcia VM (2010) Defining the molecular basis of BubR1 kinetochore interactions and APC/C-CDC20 inhibition. Journal of Biological Chemistry 285: 14764–14776. doi:10.1074/jbc.M109.082016.
154. Larsen NA, Al-Bassam J, Wei RR, Harrison SC (2007) Structural analysis of Bub3 interactions in the mitotic spindle checkpoint. Proc Natl Acad Sci USA 104: 1201–1206. doi:10.1073/pnas.0610358104.
155. Wang X, Babu JR, Harden JM, Jablonski SA, Gazi MH, et al. (2001) The mitotic checkpoint protein hBUB3 and the mRNA export factor hRAE1 interact with GLE2p-binding sequence (GLEBS)-containing proteins. J Biol Chem 276: 26559–26567. doi:10.1074/jbc.M101083200.

156. Tang Z, Bharadwaj R, Li B, Yu H (2001) Mad2-Independent inhibition of APCCdc20 by the mitotic checkpoint protein BubR1. Dev Cell 1: 227–237.
157. Davenport JW, Fernandes ER, Harris LD, Neale GA, Goorha R (1999) The mouse mitotic checkpoint gene bub1b, a novel bub1 family member, is expressed in a cell cycle-dependent manner. Genomics 55: 113–117. doi:10.1006/geno.1998.5629.
158. Musacchio A, Salmon ED (2007) The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol 8: 379–393. doi:10.1038/nrm2163.
159. Peters J-M (2006) The anaphase promoting complex/cyclosome: a machine designed to destroy. Nat Rev Mol Cell Biol 7: 644–656. doi:10.1038/nrm1988.
160. Lampson MA, Kapoor TM (2005) The human mitotic checkpoint protein BubR1 regulates chromosome-spindle attachments. Nat Cell Biol 7: 93–98. doi:10.1038/ncb1208.
161. Dai W, Wang Q, Liu T, Swamy M, Fang Y, et al. (2004) Slippage of mitotic arrest and enhanced tumor development in mice with BubR1 haploinsufficiency. Cancer Res 64: 440–445.
162. Ricke RM, van Ree JH, van Deursen JM (2008) Whole chromosome instability and cancer: a complex relationship. Trends Genet 24: 457–466. doi:10.1016/j.tig.2008.07.002.
163. Siegel JJ, Amon A (2012) New insights into the troubles of aneuploidy. Annu Rev Cell Dev Biol 28: 189–214. doi:10.1146/annurev-cellbio-101011-155807.
164. Nagaoka SI, Hassold TJ, Hunt PA (2012) Human aneuploidy: mechanisms and new insights into an age-old problem. Nat Rev Genet 13: 493–504. doi:10.1038/nrg3245.
165. Wang Q, Liu T, Fang Y, Xie S, Huang X, et al. (2004) BUBR1 deficiency results in abnormal megakaryopoiesis. Blood 103: 1278–1285. doi:10.1182/blood-2003-06-2158.
166. Holland AJ, Cleveland DW (2012) Losing balance: the origin and impact of aneuploidy in cancer. EMBO Rep 13: 501–514. doi:10.1038/embor.2012.55.
167. Gordon DJ, Resio B, Pellman D (2012) Causes and consequences of aneuploidy in cancer. Nat Rev Genet 13: 189–203. doi:10.1038/nrg3123.
168. Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JK, et al. (1998) Mutations of mitotic checkpoint genes in human cancers. Nature 392: 300–303. doi:10.1038/32688.
169. Ohshima K, Haraoka S, Yoshioka S, Hamasaki M, Fujiki T, et al. (2000) Mutation analysis of mitotic checkpoint genes (hBUB1 and hBUBR1) and microsatellite instability in adult T-cell leukemia/lymphoma. Cancer Lett 158: 141–150.
170. Rio Frio T, Lavoie J, Hamel N, Geyer FC, Kushner YB, et al. (2010) Homozygous BUB1B mutation and susceptibility to gastrointestinal neoplasia. N Engl J Med 363: 2628–2637. doi:10.1056/NEJMoa1006565.
171. Hanks S, Coleman K, Reid S, Plaja A, Firth H, et al. (2004) Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. Nat Genet 36: 1159–1161. doi:10.1038/ng1449.
172. Matsuura S, Matsumoto Y, Morishima K-I, Izumi H, Matsumoto H, et al. (2006) Monoallelic BUB1B mutations and defective mitotic-spindle checkpoint in seven families with premature chromatid separation (PCS) syndrome. Am J Med Genet A 140: 358–367. doi:10.1002/ajmg.a.31069.
173. Weaver BAA, Cleveland DW (2006) Does aneuploidy cause cancer? Curr Opin Cell Biol 18: 658–667. doi:10.1016/j.ceb.2006.10.002.
174. Ouyang B, Knauf JA, Ain K, Nacev B, Fagin JA (2002) Mechanisms of aneuploidy in thyroid cancer cell lines and tissues: evidence for mitotic checkpoint dysfunction without mutations in BUB1 and BUBR1. Clin Endocrinol (Oxf) 56: 341–350.

175. Shichiri M, Yoshinaga K, Hisatomi H, Sugihara K, Hirata Y (2002) Genetic and epigenetic inactivation of mitotic checkpoint genes hBUB1 and hBUBR1 and their relationship to survival. Cancer Res 62: 13–17.
176. Yuan B, Xu Y, Woo J-H, Wang Y, Bae YK, et al. (2006) Increased expression of mitotic checkpoint genes in breast cancer cells with chromosomal instability. Clin Cancer Res 12: 405–410. doi:10.1158/1078-0432.CCR-05-0903.
177. Grabsch H, Takeno S, Parsons WJ, Pomjanski N, Boecking A, et al. (2003) Overexpression of the mitotic checkpoint genes BUB1, BUBR1, and BUB3 in gastric cancer--association with tumour cell proliferation. J Pathol 200: 16–22. doi:10.1002/path.1324.
178. Seike M, Gemma A, Hosoya Y, Hosomi Y, Okano T, et al. (2002) The promoter region of the human BUBR1 gene and its expression analysis in lung cancer. Lung Cancer 38: 229–234.
179. Yamamoto Y, Matsuyama H, Chochi Y, Okuda M, Kawauchi S, et al. (2007) Overexpression of BUBR1 is associated with chromosomal instability in bladder cancer. Cancer Genet Cytogenet 174: 42–47. doi:10.1016/j.cancergencyto.2006.11.012.
180. Lee Y-K, Choi E, Kim MA, Park P-G, Park N-H, et al. (2009) BubR1 as a prognostic marker for recurrence-free survival rates in epithelial ovarian cancers. Br J Cancer 101: 504–510. doi:10.1038/sj.bjc.6605161.
181. Pinto M, Vieira J, Ribeiro FR, Soares MJ, Henrique R, et al. (2008) Overexpression of the mitotic checkpoint genes BUB1 and BUBR1 is associated with genomic complexity in clear cell kidney carcinomas. Cell Oncol 30: 389–395.
182. Park I, Lee H-O, Choi E, Lee Y-K, Kwon M-S, et al. (2013) Loss of BubR1 acetylation causes defects in spindle assembly checkpoint signaling and promotes tumor formation. J Cell Biol 202: 295–309. doi:10.1083/jcb.201210099.
183. Hartman TK, Wengenack TM, Poduslo JF, van Deursen JM (2007) Mutant mice with small amounts of BubR1 display accelerated age-related gliosis. Neurobiol Aging 28: 921–927. doi:10.1016/j.neurobiolaging.2006.05.012.
184. Matsumoto T, Baker DJ, d'Uscio LV, Mozammel G, Katusic ZS, et al. (2007) Aging-associated vascular phenotype in mutant mice with low levels of BubR1. Stroke 38: 1050–1056. doi:10.1161/01.STR.0000257967.86132.01.
185. Baker DJ, Weaver RL, van Deursen JM (2013) p21 both attenuates and drives senescence and aging in BubR1 progeroid mice. Cell Rep 3: 1164–1174. doi:10.1016/j.celrep.2013.03.028.
186. Limwongse C, Schwartz S, Bocian M, Robin NH (1999) Child with mosaic variegated aneuploidy and embryonal rhabdomyosarcoma. Am J Med Genet 82: 20–24.
187. García-Castillo H, Vásquez-Velásquez AI, Rivera H, Barros-Núñez P (2008) Clinical and genetic heterogeneity in patients with mosaic variegated aneuploidy: delineation of clinical subtypes. Am J Med Genet A 146A: 1687–1695. doi:10.1002/ajmg.a.32315.
188. Ochiai H, Miyamoto T, Kanai A, Hosoba K, Sakuma T, et al. (2014) TALEN-mediated single-base-pair editing identification of an intergenic mutation upstream of BUB1B as causative of PCS (MVA) syndrome. Proc Natl Acad Sci USA 111: 1461–1466. doi:10.1073/pnas.1317008111.
189. Suijkerbuijk SJE, van Osch MHJ, Bos FL, Hanks S, Rahman N, et al. (2010) Molecular causes for BUBR1 dysfunction in the human cancer predisposition syndrome mosaic variegated aneuploidy. Cancer Res 70: 4891–4900. doi:10.1158/0008-5472.CAN-09-4319.
190. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, et al. (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908: 244–254.
191. Salminen A, Kaarniranta K, Kauppinen A (2012) Inflammaging: disturbed interplay between autophagy and inflammasomes. Aging (Albany NY) 4: 166–175.

192. Csiszar A, Ungvari Z, Koller A, Edwards JG, Kaley G (2003) Aging-induced proinflammatory shift in cytokine expression profile in coronary arteries. The FASEB Journal 17: 1183–1185. doi:10.1096/fj.02-1049fje.
193. de Magalhães JP, Curado J, Church GM (2009) Meta-analysis of age-related gene expression profiles identifies common signatures of aging. Bioinformatics 25: 875–881. doi:10.1093/bioinformatics/btp073.
194. Swindell WR (2009) Genes and gene expression modules associated with caloric restriction and aging in the laboratory mouse. BMC Genomics 10: 585. doi:10.1186/1471-2164-10-585.
195. Krabbe KS, Pedersen M, Bruunsgaard H (2004) Inflammatory mediators in the elderly. Exp Gerontol 39: 687–699. doi:10.1016/j.exger.2004.01.009.
196. Singh T, Newman AB (2011) Inflammatory markers in population studies of aging. Ageing Res Rev 10: 319–329. doi:10.1016/j.arr.2010.11.002.
197. Larbi A, Franceschi C, Mazzatti D, Solana R, Wikby A, et al. (2008) Aging of the immune system as a prognostic factor for human longevity. Physiology (Bethesda) 23: 64–74. doi:10.1152/physiol.00040.2007.
198. Kovacs EJ, Palmer JL, Fortin CF, Fülöp T, Goldstein DR, et al. (2009) Aging and innate immunity in the mouse: impact of intrinsic and extrinsic factors. Trends Immunol 30: 319–324. doi:10.1016/j.it.2009.03.012.
199. Baker RG, Hayden MS, Ghosh S (2011) NF-κB, inflammation, and metabolic disease. Cell Metabolism 13: 11–22. doi:10.1016/j.cmet.2010.12.008.
200. Tabas I (2010) Macrophage death and defective inflammation resolution in atherosclerosis. Nat Rev Immunol 10: 36–46. doi:10.1038/nri2675.
201. Doles J, Storer M, Cozzuto L, Roma G, Keyes WM (2012) Age-associated inflammation inhibits epidermal stem cell function. Genes & Development 26: 2144–2153. doi:10.1101/gad.192294.112.
202. Deeks SG (2011) HIV infection, inflammation, immunosenescence, and aging. Annu Rev Med 62: 141–155. doi:10.1146/annurev-med-042909-093756.
203. Hayden MS, Ghosh S (2008) Shared Principles in NF-κB Signaling. Cell 132: 344–362. doi:10.1016/j.cell.2008.01.020.
204. Hayden MS, West AP, Ghosh S (2006) NF-kappaB and the immune response. Oncogene 25: 6758–6780. doi:10.1038/sj.onc.1209943.
205. Hayden MS, Ghosh S (2012) NF-κB, the first quarter-century: remarkable progress and outstanding questions. Genes & Development 26: 203–234. doi:10.1101/gad.183434.111.
206. Tilstra JS, Clauson CL, Niedernhofer LJ, Robbins PD (2011) NF-κB in Aging and Disease. Aging Dis 2: 449–465.
207. Kriete A, Mayo KL, Yalamanchili N, Beggs W, Bender P, et al. (2008) Cell autonomous expression of inflammatory genes in biologically aged fibroblasts associated with elevated NF-kappaB activity. Immun Ageing 5: 5. doi:10.1186/1742-4933-5-5.
208. Adler AS, Sinha S, Kawahara TLA, Zhang JY, Segal E, et al. (2007) Motif module map reveals enforcement of aging by continual NF-kappaB activity. Genes & Development 21: 3244–3257. doi:10.1101/gad.1588507.
209. Helenius M, Hänninen M, Lehtinen SK, Salminen A (1996) Changes associated with aging and replicative senescence in the regulation of transcription factor nuclear factor-kappa B. Biochem J 318 (Pt 2): 603–608.
210. Korhonen P, Helenius M, Salminen A (1997) Age-related changes in the regulation of transcription factor NF-kappa B in rat brain. Neurosci Lett 225: 61–64.

211. Giardina C, Hubbard AK (2002) Growing old with nuclear factor-kappaB. Cell Stress Chaperones 7: 207–212.
212. Cai D, Frantz JD, Tawa NE, Melendez PA, Oh B-C, et al. (2004) IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. Cell 119: 285–298. doi:10.1016/j.cell.2004.09.027.
213. Ghosh A, Roy A, Liu X, Kordower JH, Mufson EJ, et al. (2007) Selective inhibition of NF-kappaB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. Proc Natl Acad Sci USA 104: 18754–18759. doi:10.1073/pnas.0704908104.
214. Cuaz-Pérolin C, Billiet L, Baugé E, Copin C, Scott-Algara D, et al. (2008) Antiinflammatory and antiatherogenic effects of the NF-kappaB inhibitor acetyl-11-keto-beta-boswellic acid in LPS-challenged ApoE-/- mice. Arterioscler Thromb Vasc Biol 28: 272–277. doi:10.1161/ATVBAHA.107.155606.
215. Karin M (2006) Nuclear factor-kappaB in cancer development and progression. Nature 441: 431–436. doi:10.1038/nature04870.
216. Mostoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, et al. (2006) Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell 124: 315–329. doi:10.1016/j.cell.2005.11.044.
217. Kawahara TLA, Michishita E, Adler AS, Damian M, Berber E, et al. (2009) SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. Cell 136: 62–74. doi:10.1016/j.cell.2008.10.052.
218. Niedernhofer LJ, Garinis GA, Raams A, Lalai AS, Robinson AR, et al. (2006) A new progeroid syndrome reveals that genotoxic stress suppresses the somatotroph axis. Nature 444: 1038–1043. doi:10.1038/nature05456.
219. Tilstra JS, Robinson AR, Wang J, Gregg SQ, Clauson CL, et al. (2012) NF-κB inhibition delays DNA damage-induced senescence and aging in mice. J Clin Invest 122: 2601–2612. doi:10.1172/JCI45785.
220. Perkins ND (2006) Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene 25: 6717–6730. doi:10.1038/sj.onc.1209937.
221. Huang B, Yang X-D, Lamb A, Chen L-F (2010) Posttranslational modifications of NF-kappaB: another layer of regulation for NF-kappaB signaling pathway. Cell Signal 22: 1282–1290. doi:10.1016/j.cellsig.2010.03.017.
222. Zhong H, SuYang H, Erdjument-Bromage H, Tempst P, Ghosh S (1997) The transcriptional activity of NF-kappaB is regulated by the IkappaB-associated PKAc subunit through a cyclic AMP-independent mechanism. Cell 89: 413–424.
223. Jamaluddin M, Wang S, Boldogh I, Tian B, Brasier AR (2007) TNF-alpha-induced NF-kappaB/RelA Ser(276) phosphorylation and enhanceosome formation is mediated by an ROS-dependent PKAc pathway. Cell Signal 19: 1419–1433. doi:10.1016/j.cellsig.2007.01.020.
224. Vermeulen L, De Wilde G, Van Damme P, Vanden Berghe W, Haegeman G (2003) Transcriptional activation of the NF-kappaB p65 subunit by mitogen- and stress-activated protein kinase-1 (MSK1). EMBO J 22: 1313–1324. doi:10.1093/emboj/cdg139.
225. Zhong H, Voll RE, Ghosh S (1998) Phosphorylation of NF-kappa B p65 by PKA stimulates transcriptional activity by promoting a novel bivalent interaction with the coactivator CBP/p300. Mol Cell 1: 661–671.
226. Zhong H, May MJ, Jimi E, Ghosh S (2002) The phosphorylation status of nuclear NF-kappa B determines its association with CBP/p300 or HDAC-1. Mol Cell 9: 625–636.

227. Dong J, Jimi E, Zhong H, Hayden MS, Ghosh S (2008) Repression of gene expression by unphosphorylated NF-κB p65 through epigenetic mechanisms. Genes & Development 22: 1159–1173. doi:10.1101/gad.1657408.
228. Dong J, Jimi E, Zeiss C, Hayden MS, Ghosh S (2010) Constitutively active NF-κB triggers systemic TNFα-dependent inflammation and localized TNFα-independent inflammatory disease. Genes & Development 24: 1709–1717. doi:10.1101/gad.1958410.
229. Mao X, Gluck N, Li D, Maine GN, Li H, et al. (2009) GCN5 is a required cofactor for a ubiquitin ligase that targets NF-κB/RelA. Genes & Development 23: 849–861. doi:10.1101/gad.1748409.
230. Geng H, Wittwer T, Dittrich-Breiholz O, Kracht M, Schmitz ML (2009) Phosphorylation of NF-κB p65 at Ser468 controls its COMMD1-dependent ubiquitination and target gene-specific proteasomal elimination. EMBO Rep 10: 381–386. doi:10.1038/embor.2009.10.
231. Buss H (2004) Phosphorylation of Serine 468 by GSK-3β Negatively Regulates Basal p65 NF-κB Activity. Journal of Biological Chemistry 279: 49571–49574. doi:10.1074/jbc.C400442200.
232. Schwabe RF (2005) IKKβ phosphorylates p65 at S468 in transactivaton domain 2. The FASEB Journal. doi:10.1096/fj.05-3736fje.
233. Mattioli I, Geng H, Sebald A, Hodel M, Bucher C, et al. (2006) Inducible Phosphorylation of NF-κB p65 at Serine 468 by T Cell Costimulation Is Mediated by IKK. Journal of Biological Chemistry 281: 6175–6183. doi:10.1074/jbc.M508045200.
234. Moreno R, Sobotzik JM, Schultz C, Schmitz ML (2010) Specification of the NF-κB transcriptional response by p65 phosphorylation and TNF-induced nuclear translocation of IKK. Nucleic Acids Research 38: 6029–6044. doi:10.1093/nar/gkq439.