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Vascular Cell Senescence: Contribution to Atherosclerosis

Telomere Biology and Cardiovascular Disease

Mechanisms of Cardiovascular Disease in Accelerated Aging Syndromes

Progenitor Cell Senescence

Mechanisms Underlying Caloric Restriction, Lipid Metabolism, and Life Span Regulation *Issei Komuro, Guest Editor*

Vascular Cell Senescence Contribution to Atherosclerosis

Tohru Minamino, Issei Komuro

Abstract—Cardiologists and most physicians believe that aging is an independent risk factor for human atherosclerosis, whereas atherosclerosis is thought to be a characteristic feature of aging in humans by many gerontologists. Because atherosclerosis is among the age-associated changes that almost always escape the influence of natural selection in humans, it might be reasonable to regard atherosclerosis as a feature of aging. Accordingly, when we investigate the pathogenesis of human atherosclerosis, it may be more important to answer the question of how we age than what specifically promotes atherosclerosis. Recently, genetic analyses using various animal models have identified molecules that are crucial for aging. These include components of the DNA-repair system, the tumor suppressor pathway, the telomere maintenance system, the insulin/Akt pathway, and other metabolic pathways. Interestingly, most of the molecules that influence the phenotypic changes of aging also regulate cellular senescence, suggesting a causative link between cellular senescence and aging. For example, DNA-repair defects can cause phenotypic changes that resemble premature aging, and senescent cells that show DNA damage accumulate in the elderly. Excessive calorie intake can cause diabetes and hyperinsulinemia, whereas dysregulation of the insulin pathway has been shown to induce cellular senescence in vitro. Calorie restriction or a reduction of insulin signals extends the lifespan of various species and decreases biomarkers of cellular senescence in vivo. There is emerging evidence that cellular senescence contributes to the pathogenesis of human atherosclerosis. Senescent vascular cells accumulate in human atheroma tissues and exhibit various features of dysfunction. In this review, we examine the hypothesis that cellular senescence might contribute to atherosclerosis, which is a characteristic of aging in humans. (*Circ Res.* 2007;100:15-26.)

Key Words: p53 ■ insulin ■ diabetes ■ angiotensin II ■ telomere

Epidemiological studies have shown that age is the dominant risk factor for atherosclerotic cardiovascular diseases.^{1,2} The incidence and the prevalence of atherothrombotic diseases, including coronary heart disease and stroke, both increase with advancing age.^{1,2} However, the molecular mechanisms underlying the increased risk of such diseases that is conferred by aging remain unclear. For example,

arterial stiffness increases with age because of structural changes of the arterial walls as well as endothelial dysfunction, but convincing molecular explanations for these age-associated alterations of vascular structure and function have not yet been reported. Because aging includes various biological phenomena, it is difficult to attribute age-related changes of the vasculature or the organism to a certain

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molecule. Moreover, there are no accurate biomarkers for aging, which makes it problematic to study vascular aging.

Cellular senescence is originally described as the finite replicative lifespan of human somatic cells in culture. Senescent cells enter an irreversible growth arrest, exhibit a flattened and enlarged morphology, and express a different set of genes, including negative regulators of the cell cycle such as p53 and p16. These phenotypic changes of senescent cells are not observed in quiescent cells and have been implicated in aging and age-associated diseases.^{3,4} Primary cultured cells from patients with premature aging syndromes, such as Werner syndrome and Bloom syndrome, are known to have a shorter lifespan than cells from age-matched healthy persons,^{5,6} supporting the notion that cellular senescence is associated with aging. This hypothesis of cellular aging was first described by Hayflick in the 1960s.^{7,8} However, the role of cellular senescence in human atherosclerosis has not attracted much attention in the past, and controversy exists regarding the relationship between cellular senescence and age-related diseases.

Recently, some research groups, including ours, have reexamined the hypothesis that vascular cell senescence might contribute to the pathogenesis of age-associated vascular diseases such as atherosclerosis by using cellular senescence as a model of aging in vivo. This approach may allow us to identify important molecules and signaling pathways involved in vascular aging. Although atherosclerosis has been considered as a chronic inflammatory disease and a number of underlying mechanisms have been implicated,⁹ accumulating evidence has suggested a critical role of vascular cell senescence in atherogenesis. In the present review, we describe recent evidence that supports the hypothesis of cellular aging in the vasculature and discuss the potential of antisenescence therapy for treating human atherosclerosis.

Does Vascular Cell Senescence Induce Vascular Dysfunction Associated With Aging?

Age-associated changes of the blood vessels include a decrease in compliance and an increase of inflammation.¹⁰ It has also been reported that angiogenesis becomes impaired with advancing age^{11–13} and that aging decreases the antithrombotic properties of the endothelium.^{14,15} These changes of vascular structure and function have been suggested to have a role in the increased risk of atherosclerotic cardiovascular disease in the elderly.^{1,16} A number of studies have shown that many of the changes of senescent vascular cells are consistent with those seen in human atherosclerosis (Table in the online data supplement, available at <http://circres.ahajournals.org>), suggesting a critical role of cellular senescence in vascular pathophysiology. Both NO production and endothelial NO synthase activity are reduced in senescent human vascular endothelial cells.^{17–19} The increment of NO production in response to shear stress is also less in senescent vascular endothelial cells.¹⁸ Production of reactive oxygen species (ROS) is increased in senescent cells,^{20–25} leading to a decrease in the bioavailability of NO and increased formation of peroxynitrite.²⁶ Furthermore, prostacyclin production shows a significant decrease, with in vitro aging of vascular

endothelial cells, whereas production of thromboxane A₂ and endothelin-1 is augmented.^{27–29} Finally, senescent vascular endothelial cells show the upregulation of plasminogen activator inhibitor-1.^{30–32} All of these changes are likely to be involved in the impairment of endothelium-dependent vasodilation, as well as the increased tendency for thrombogenesis that occur in human atherosclerosis.

The interaction between monocytes and vascular endothelial cells is enhanced by endothelial cell senescence,³³ also promoting atherogenesis. This change appears to be mediated by upregulation of adhesion molecules and proinflammatory cytokines, as well as through decreased production of NO by senescent endothelial cells.^{18,19,34,35} It has been reported that senescent endothelial cells show a reduced ability to form capillaries in vitro.³⁶ Bone marrow–derived circulating endothelial progenitor cells (EPCs) are known to participate in postnatal neovascularization and vascular repair.^{37–39} The growth and function of cultured bone marrow–derived EPCs are impaired in patients with coronary artery disease and show a negative correlation with various risk factors for coronary atherosclerosis, including age.^{40,41} Thus, aging may promote the senescence of EPCs as well as vascular endothelial cells, resulting in a decline of angiogenesis and vascular healing.

There is also evidence indicating that senescence-associated functional changes occur in vascular smooth muscle cells (VSMCs). The in vitro response of VSMCs to NO and β -adrenoreceptor stimulation is decreased by aging, and such changes may contribute to impairment of endothelium-dependent (as well as endothelium-independent) vasodilation in the elderly.^{10,42} Production of elastase by senescent VSMCs is increased,⁴³ whereas expression of fibronectin is enhanced in senescent vascular endothelial cells,^{44,45} as seen in the arteries of the elderly, and such changes may be relevant to age-dependent modifications of the extracellular matrix. It has been reported that senescent fibroblasts are resistant to apoptosis.⁴⁶ In contrast to fibroblasts, apoptosis is enhanced in senescent vascular endothelial cells,^{47,48} a change that may contribute to plaque erosion and thrombosis in human atherosclerosis.⁴⁹ Circadian rhythms are regulated by a set of clock genes that form transcriptional feedback loops and generate circadian oscillation with a 24-hour cycle. The circadian rhythm of blood pressure is often impaired with advancing age, so that there is no decrease at night (non-dipper status), and this change is known to increase the risk of cardiovascular disease.^{50,51} A recent study showed that circadian expression of clock genes is impaired by cellular aging both in vitro and in vivo, suggesting that cellular senescence in peripheral tissues may underlie the mechanism by which aging impairs the entrainment of peripheral circadian rhythms.⁵²

In Vivo Evidence of Vascular Cell Senescence

Vascular cells derived from human atherosclerotic plaques showed impaired growth in vitro and undergo senescence earlier than cells from normal vessels.^{44,53,54} The histology of human atherosclerotic lesions has been extensively studied, and it has been demonstrated that these lesions contain endothelial cells and VSMCs that exhibit the morphological

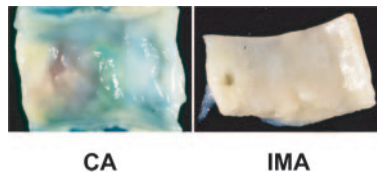


Figure 1. SA β -gal-positive vascular cells in human atheroma. Photographs of the luminal surface of human coronary artery (CA) (left) and internal mammary artery (IMA) (right) stained with β -gal staining. Senescent-associated β -gal activity was observed in human coronary arteries but not in internal mammary arteries. Adapted from Minamino et al¹⁹ by permission of the American Heart Association ©2002.

features of senescence.^{55,56} These findings suggest the occurrence of cellular senescence *in vivo*. Primary cultured cells undergoing senescence *in vitro* show increased activity of β -galactosidase (β -gal) when assayed at pH 6, which is distinguishable from endogenous lysosomal β -gal activity, which can be detected at pH 4. The former type is known as senescence-associated (SA) β -gal activity and has been shown to correlate with cellular aging.⁵⁷

Fenton et al successfully detected SA β -gal-positive vascular cells in injured rabbit carotid arteries.⁵⁸ With repeated endothelial denudation, the accumulation of SA β -gal-positive cells was markedly enhanced. SA β -gal-positive vascular cells have also been demonstrated in human atherosclerotic plaques obtained from the coronary arteries of patients with ischemic heart disease.^{19,59} These cells are predominantly localized on the luminal surface of the plaque and have been identified as endothelial cells, whereas no SA β -gal-positive cells are observed in the internal mammary arteries of the same patients in whom atherosclerotic changes are minimal (Figure 1).¹⁹ In advanced plaques, however, SA β -gal-positive VSMCs are only detected in the intima and not in the media,⁶⁰ which may be the result of extensive replication in these lesions, as observed in arteries subjected to double endothelial denudation. SA β -gal-positive cells in human atheroma show enhanced expression of p53 and p16. Because expression of these molecules is increased in senescent cells *in vitro*, these observations further suggest the *in vivo* occurrence of vascular cell senescence.⁶⁰ These cells also exhibit various functional abnormalities, such as decreased expression of endothelial NO synthase and increased expression of proinflammatory molecules.⁶⁰ Although SA β -gal activity may not be a strict biomarker of senescence, these findings suggest that cellular senescence may contribute to the pathogenesis of vascular aging.

Molecular Mechanism of Cellular Senescence

Over the past few decades, significant progress has been made in our understanding of the mechanisms underlying cellular senescence. One widely discussed hypothesis of senescence is the telomere hypothesis.⁶¹ Telomeres are non-nucleosomal DNA/protein complexes located at the ends of chromosomes that serve as protective caps and act as the substrate for specialized replication mechanisms. As a consequence of semiconservative DNA replication, the extreme terminals of the chromosomes are not duplicated completely, resulting in successive shortening of the telomeres with each

cell division. Critical telomere shortening is thought to trigger the onset of cellular senescence. Thus, telomere shortening has been proposed to act as a mitotic clock that prevents unlimited proliferation of human somatic cells.

Telomerase is a ribonucleoprotein that adds telomeres to the ends of chromosomes using its RNA moiety as a template. Since early studies reported that telomerase activity was detected in cancer cells and stem cells but not in normal somatic cells, the idea emerged that telomerase activity might be essential for tumor growth and the self-renewal potential of stem cells.^{62,63} Increasing evidence has suggested that telomerase activity regulates cell proliferation in normal somatic cells by telomere lengthening or by telomere length-independent mechanisms.^{64–66} Human endothelial cells and VSMCs express telomerase activity, which is drastically activated by mitogenic stimuli via a protein kinase C-dependent pathway,⁶⁷ but the activity declined with *in vitro* aging because of a decrease in expression of TERT, leading to telomere shortening and cellular senescence.^{68,69} Introduction of telomerase extends the lifespan of both endothelial cells and VSMCs,^{36,68,70} suggesting a critical role of telomere and telomerase in vascular cell senescence.

It is likely that excessively short telomeres are sensed by cells, resulting in a pathway being activated that leads to exit from the cell cycle. It is now apparent that cellular senescence can also be induced by various kinds of stress independently of the replicative age of a cell.^{4,71} Cells undergo senescence by DNA damage that is irreparable or threatens to overwhelm the DNA-repair machinery.⁷² Supraphysiological mitogenic signals that result from the overexpression of oncogenes also elicit senescence in many normal cells.⁷¹ Finally, cells can undergo senescence in response to epigenetic changes of chromatin organization that may alter the expression of protooncogenes or tumor suppressor genes.⁷³ Thus, it is possible that atherogenic stimuli increase cell turnover at sites of atherosclerosis, thereby promoting telomere shortening and possibly also activating certain proliferative signals that may induce senescence independently of telomere shortening. Moreover, oxidative stress and DNA damage could increase vascular cell senescence and thus further promote atherogenesis.^{74,75}

Although diverse stimuli can induce senescence, they appear to converge mainly on either or both of 2 pathways that establish and maintain the process of cellular senescence. These pathways are regulated by the tumor suppressor proteins p53 and pRb.^{71,76} Both proteins are transcriptional regulators, and each lies at the center of signaling pathways responsible for cell cycle regulation, DNA repair, and cell death, which involve a number of upstream regulators and downstream effectors.⁷⁷ p53 is a crucial mediator of the cellular response to DNA damage, and it induces the cyclin-dependent inhibitor p21.⁷⁸ Dysfunctional telomeres resemble damaged DNA and thus trigger a p53-dependent response.⁷⁹ Recent studies have demonstrated that nuclear foci containing markers of double-strand DNA breaks form in cells with critically short or dysfunctional telomeres,^{80,81} and it has been shown that such telomere dysfunction-induced nuclear foci are increased in the fibroblasts of aging primates.⁸² The p53 pathway is also important for senescence occurring in re-

sponse to oncogenic stimuli such as activation of Ras.^{83–85} Oncogenic Ras may trigger a p53-dependent damage response by increasing the production of ROS, which are required for the mitogenic effects of Ras activation.²²

p16 is a positive regulator of pRb and induced by a variety of stress stimuli, including the overexpression of oncogenes such as Ras.⁸⁶ In some cells, p16 expression is silenced by the methylation of its promoter,^{87,88} and the senescence response depends primarily on the p53-dependent pathway in such cells. However, at least some cells (including vascular cells) show increased expression of p16 during replicative senescence.^{4,89} Loss of p16 combined with retention of the p53-dependent pathway predisposes mice to tumorigenesis.⁹⁰ Overexpression of p16 has been shown to prevent the reversal of senescence caused by inactivation of p53.⁹¹ Thus, the p16/pRb pathway appears to provide a formidable barrier to cell proliferation that cannot be overcome by loss of p53. It has been reported that the p16/pRb pathway induces reorganization of chromatin and thus affects the expression of cell cycle regulators.^{92–94} Senescent cells develop dense foci of heterochromatin that repress the E2F target genes encoding positive cell cycle regulators in a pRb-dependent manner.^{92–94} Although the whole picture of cellular senescence regulated by cell cycle modulators is far from complete and the questions of how many subpathways are involved have not yet been fully addressed, it is generally suggested that senescence occurs via the p53 pathway in response to DNA damage and telomere dysfunction, whereas the p16/pRb pathway mediates senescence caused by oncogenic stimuli, chromatin disruption, and other cellular stresses.

Telomere-Dependent Senescence Impairs Vascular Function

There is evidence indicating that telomere shortening occurs in human vessels, and this may be related to age-associated vascular diseases. The telomere length in the endothelial cells of the abdominal aorta and iliac arteries shows a strong inverse correlation with age.^{70,95} Importantly, telomere shortening occurs faster in the endothelial cells of the iliac arteries compared with those of the internal mammary arteries.⁷⁰ Thus, high levels of hemodynamic stress may enhance the rate of endothelial cell turnover in the iliac arteries compared with that in vessels subjected to low hemodynamic stress. Telomere shortening is also more advanced in coronary artery endothelial cells from patients with coronary heart disease compared with cells from healthy subjects.⁹⁶

The telomere length of white blood cells from healthy subjects shows an inverse correlation with the pulse pressure that is independent of the chronological age, at least in men.⁹⁷ In patients with severe coronary artery disease, the telomeres of white blood cells are significantly shorter than those of cells from controls, which might reflect accelerated biological aging of various tissues (including the coronary arteries).⁹⁸ In fact, the risk of myocardial infarction is increased by approximately 2- to 3-fold in subjects with shorter telomeres.⁹⁹ Shorter telomeres are associated with an increase of carotid atherosclerosis in hypertensive subjects,¹⁰⁰ whereas degenerative aortic valve stenosis is correlated with telomere shortening in the elderly, and this correlation is independent of

coronary heart disease.¹⁰¹ Short telomeres are also found in patients with vascular dementia.¹⁰² It has been reported that various risk factors for cardiovascular disease, such as obesity, smoking, psychological stress, insulin resistance, hypertension, and diabetes, are associated with lower telomerase activity or shortening of the telomeres in white blood cells.^{103–108} Cawthon et al¹⁰⁹ examined the telomere length in 143 normal unrelated subjects who were more than 60 years of age and found that those with shorter telomeres in their white blood cells had worse survival, which was attributable to a 3.18-fold higher mortality rate from heart disease and an 8.54-fold higher mortality rate from infections. In contrast, 2 recent studies demonstrated no association between telomere length and mortality in the elderly.^{110,111}

It has been demonstrated that disturbance of telomere integrity leads to endothelial dysfunction *in vitro*.¹⁹ Telomerase-deficient mice have a normal phenotype in the first generation, presumably because mice possess very long telomeres.^{64,112} However, their telomeres become shorter with successive generations, and these mice become infertile by the sixth generation because of impairment of the reproductive system.⁶⁴ Some of the changes in the later generations of these mice mimic age-associated phenomena. For example, these animals have a shortened lifespan and a reduced capacity to respond to stresses such as wounds and hematopoietic ablation.¹¹³ Neovascularization is also impaired in the later generations of telomerase-deficient mice.¹¹⁴ This decreased ability to form new vessels may be attributable to impairment of the function and replicative capacity of vascular endothelial cells induced by telomere shortening. In a mouse model of atherosclerosis, telomere shortening has been shown to decrease the area of atherosclerotic lesions, presumably because of reduced proliferation of macrophages.¹¹⁵ However, telomerase-deficient mice develop atherosclerotic plaques with a thin fibrous cap, suggesting that shortening of the telomeres in vascular cells may induce plaque rupture in human atherosclerosis. Mice lacking telomerase activity develop hypertension in the first and third generations as a result of an increase in plasma endothelin-1 levels caused by endothelin-converting enzyme overexpression.¹¹⁶ In the spontaneously hypertensive rats (SHRs), a widely used model of essential hypertension, vascular hypertrophy develops before there is significant elevation of the blood pressure, and is associated with an increase in both the number and size of VSMCs. In contrast to telomerase-deficient mice, Cao et al¹¹⁷ demonstrated that telomerase activity was increased in the aortas of SHRs compared with normotensive control rats. This increase of activity was detected as early as 3 weeks after birth, which was well before the onset of hypertension. Likewise, telomerase activity was increased in injured arteries, and inhibition of the activity reduced intimal formation after injury, suggesting that increased telomerase activity promotes vascular remodeling and contributes to the elevation of blood pressure.^{67,118} Although telomerase-deficient mouse is not strictly a model of aging, further studies using this model will provide insights into the role of telomere and telomerase in vascular pathophysiology.

Angiotensin II Induces Premature Vascular Cell Senescence

Arterial components of the angiotensin II (Ang II) signaling cascade increase with aging and contribute to the pathogenesis of atherosclerosis, and inhibition of Ang II activity has been demonstrated to improve the morbidity and mortality of cardiovascular disease.¹¹⁹ Ang II signaling appears to play a critical role in regulating many of the stimuli and signals that govern vascular aging and atherogenesis. Recently, Ang II was reported to induce the premature senescence of human VSMCs via the p53/p21-dependent pathway.⁷⁴ Inhibition of this pathway effectively suppresses the induction of proinflammatory cytokine production, as well as premature senescence, in VSMCs by Ang II. Ang II was also shown to increase the number of senescent VSMCs and induce the expression of proinflammatory molecules, as well as p21, in a mouse model of atherosclerosis.⁷⁴ Loss of p21 markedly ameliorated the induction of proinflammatory molecules by Ang II, thereby preventing the development of atherosclerosis. Merched and Chan¹²⁰ also reported that lack of p21 has a protective effect against atherosclerosis in fat-fed apolipoprotein E (apoE)-deficient mice.

p53 immunoreactivity is detectable in vascular cells from sites of chronic inflammation in human atheroma, whereas only a few cells are positive for p53 in control arteries.^{60,121} p21 immunoreactivity is also detected in human atheroma, but not in normal vessels, and it colocalizes with p53. The overexpression of cyclin-dependent kinase inhibitors induces premature senescence of cultured vascular cells that is associated with cellular dysfunction.⁷⁴ These observations suggest a pathological role of p53 and p21 in atherogenesis. It has been demonstrated that atherosclerosis is aggravated in p53/apoE double-knockout mice through an increase of p53-controlled proliferation.¹²² Moreover, apoE*3–Leiden transgenic mice, a variant of the apoE knockout mouse, undergo accelerated atherosclerosis after bone marrow reconstitution with p53-deficient marrow cells.¹²³ In contrast, a study using a perivascular collar in apoE knockout mice shows that p53 overexpression resulted in a marked decrease of the cellular and extracellular contents of the cap region, leading to spontaneous plaque rupture,¹²⁴ whereas p21 deficiency stabilized atherosclerotic plaques by inhibiting vascular inflammation and inducing VSMC growth to prevent plaque rupture.⁷⁴ Thus, overexpression of p53 and p21 by vascular cells may have a deleterious effect in human atherosclerosis.

Ang II is an important molecule that activates the Ras signaling pathway. Consistent with the influence of Ang II on senescence, constitutive activation of Ras has been found to induce vascular cell senescence and is associated with vascular inflammation.⁶⁰ Activation of Ras leads to a marked increase in the expression of proinflammatory cytokines by cultured vascular cells, which is partially mediated through extracellular signal-regulated kinase activation. Introduction of Ras into balloon-injured arteries enhances vascular inflammation, as well as cellular senescence, compared with the responses seen in control injured arteries. Moreover, senescent cells in human atherosclerotic plaque express inflammatory molecules, and extracellular signal-regulated kinase is activated in these cells, suggesting that a Ras-dependent

mechanism may also contribute to vascular cell senescence in human atherosclerosis. Consistent with these findings, inhibition of Ras activity has been demonstrated to suppress the release of proinflammatory molecules, thereby reducing lesion formation in apoE-deficient mice.¹²⁵ Thus, the Ang II/Ras signaling pathway appears to promote atherogenesis in humans at least partially by inducing vascular cell senescence via the p53/p21 pathway.

Oxidative Stress, Mitochondria, and DNA Damage

Oxidative stress has been suggested to have a role in human aging as well as cellular senescence.¹²⁶ Chronic oxidative stress caused by chemical oxidants induces telomere shortening and accelerates the onset of senescence,¹²⁷ as well as senescence-associated death,²⁵ in human endothelial cells. Homocysteine, which is among the risk factors for atherosclerosis, induces telomere shortening and accelerates endothelial cell senescence.¹²⁸ Oxidized low-density lipoprotein is reported to inactivate telomerase by inhibiting the phosphoinositol 3-kinase/Akt pathway in vascular endothelial cells¹²⁹ and induces premature senescence.¹³⁰ Conversely, suppression of oxidative stress or hypoxia preserves telomere length and extends the lifespan of cells, at least partly through an increase of telomerase activity.^{23,68,131} NO also activates telomerase and delays the onset of endothelial cell senescence. It is possible that NO reacts with intracellular radicals and decreases oxidative stress, resulting in the activation of telomerase.¹³² Constitutive activation of the small GTPase rac-1 results in mitochondrial oxidative stress via the generation of ceramide and thus induces premature endothelial cell senescence.²⁴ Ceramide also increases when endothelial cells undergo replicative senescence.^{47,133} Senescence-enhanced oxidative stress appears to be associated with decreased expression of mitochondrial cytochrome *c* oxidase by vascular endothelial cells.^{20,134} Altered expression of other mitochondrial genes has also been reported and may participate in endothelial cell senescence.¹³⁵ Vascular endothelial cells cultured on a glycated type I collagen substrate undergo early senescence, and antioxidant treatment delays the onset of senescence,¹³⁶ which may be related to the pathogenesis of diabetic vasculopathy. Asymmetrical dimethyl L-arginine (ADMA), an endogenous NO synthesis inhibitor, increases cellular ROS levels and accelerates endothelial cell senescence.¹³⁷ Conversely, aspirin reduces the formation of ADMA, decreases ROS levels and increases NO production, thereby delaying the onset of endothelial cell senescence.¹³⁸ Treatment with L-arginine effectively inhibits ADMA- or homocysteine-accelerated endothelial cell aging by increasing NO formation and consequently the induction of heme oxygenase-1.¹³⁹ Induction of senescence-like phenotypic changes has been reported in vascular endothelial cells exposed to ionizing radiation.¹⁴⁰

The majority of ROS can be traced back to the mitochondria. The generation of mitochondrial ROS is a consequence of oxidative phosphorylation, a process that produces a potential energy for protons across the mitochondrial inner membrane. A number of studies have demonstrated that mitochondrial integrity declines with aging.¹⁴¹ It has been

also reported that point mutations or deletions of mitochondrial DNA progressively accumulates during human aging. This accumulation is postulated to lead to the promotion of mitochondrial dysfunction and the generation of ROS that, in turn, induces further mitochondrial DNA mutations. There is evidence suggesting the importance of mitochondrial dysfunction in cellular senescence.¹⁴² Increased ROS caused by mitochondrial dysfunction is thought to induce telomere shortening and accelerate the onset of senescence. Interestingly, vascular complications are commonly observed in young subjects with mitochondrial disease in the absence of risk factors for atherosclerosis.¹⁴³ Indeed, mitochondrial DNA damage correlates with the extent of atherosclerosis,¹⁴⁴ suggesting that mitochondrial dysfunction may promote atherogenesis by inducing vascular cell senescence.

Recently, Trifunovic et al have provided evidence that supports the mitochondrial DNA mutation theory for aging. They generated mitochondrial mutator mice that express a proof-reading-deficient version of mitochondrial DNA polymerase. These mice display a normal phenotype at birth and in early adolescence but subsequently acquire many features of premature aging.¹⁴⁵ Conversely, overexpression of catalase in the mitochondria reduces oxidative damage, extends murine lifespan, and delays the onset of vascular aging.¹⁴⁶ However, more recent studies have shown that mitochondrial DNA mutations cause aging phenotypes without affecting ROS production.^{147,148} Thus, a causal relationship between mitochondrial DNA mutations, oxidative stress, and vascular aging remains to be fully determined.

One of the cellular targets of ROS is DNA. Many different types of oxidative DNA lesions have been described, ranging from base modifications to single- and double-strand breaks.¹⁴⁹ To cope with DNA damage, cells have evolved DNA-repair systems. Breaks that affect only 1 DNA strand are repaired via the processes of nucleotide-excision repair or base-excision repair, whereas double-strand breaks are repaired by homologous recombination or by nonhomologous recombination with end joining. Some mice that lack components of these DNA-repair systems exhibit the early onset of changes associated with aging, like their human counterparts,^{72,150–157} and fibroblasts from these mice show accelerated senescence. Constitutive activation of p53,^{158,159} but not an increase of p53 activity mediated through an extra copy of the intact p53 gene,¹⁶⁰ causes premature aging that is characterized by a reduced lifespan, osteoporosis, organ atrophy, and diminished stress tolerance. More importantly, cellular senescence has been detected in vivo by studies of mice with premature aging.¹⁶¹ Thus, these results provide in vivo evidence linking cellular senescence to aging of the organism. However, there are no reports that mice with premature aging show accelerated atherosclerosis. Expression of p16 increases in almost all tissues with advancing age and is considered as a biomarker of aging.¹⁶² Recently, the separate groups examined its role in regeneration of 3 different tissues, the blood, pancreas, and brain, and found that p16 is not only a biomarker but an effector of aging.^{163–165} However, it remains unclear whether p16 contributes to the pathogenesis of vascular aging.

Premature Aging Syndromes

Among the human progeroid syndromes, patients with Werner syndrome (WRN) or Hutchinson–Gilford progeria syndrome (HGPS) prematurely develop atherosclerosis and often die of myocardial infarction.^{6,149} The mutation causing WRN has been identified as affecting a member of the RecQ family of helicases. WRN helicase appears to be involved in DNA recombination, replication, repair, and transcription, as well as in maintaining telomere integrity. WRN-null mice develop normally and do not exhibit premature aging.¹⁶⁶ In contrast, WRN-deficient mice that have shorter telomeres like humans show a variety of changes similar to those seen in WRN patients,^{167,168} suggesting that telomere attrition is a key element in the pathology of WRN. HGPS is referred to as “childhood progeria” to differentiate it from WRN, which is referred to as “adult progeria.” Patients with HGPS progressively develop atherosclerosis, and myocardial infarction or cerebrovascular events occur at an average age of 13 years.⁶ Mutations causing HGPS have been identified in the human nuclear lamin A gene.^{169,170} It has been suggested that accumulation of mutant lamin A causes progressive changes of nuclear architecture and epigenetic control of gene expression, thus inducing premature aging.^{171–173} Like WRN fibroblasts, cultured fibroblasts obtained from HGPS patients undergo early senescence.¹⁷¹ Thus, it is assumed that accelerated senescence of vascular cells may underlie the occurrence of premature atherosclerosis in these diseases. However, because the changes of vascular aging have not been described in the relevant mouse models, more studies are necessary to clarify our understanding of the mechanism of premature atherosclerosis.

Vascular Cell Senescence in Diabetic Vasculopathy

Restriction of calorie intake is known to improve the longevity of organisms ranging from yeast to mice. Low calorie intake prevents various age-dependent changes, such as the development of cancer and atherosclerosis, the decline of immunity, and the increase of inflammatory parameters.^{174–176} Because the plasma levels of glucose, insulin, and insulin-like growth factor-1 are decreased by calorie restriction, changes of these pathways may underlie the improvement of longevity related to a lower calorie intake.¹⁷⁴ Consistent with these observations, genetic analyses have demonstrated that reduction-of-function mutations of the signaling pathway for insulin/insulin-like growth factor-1/phosphatidylinositol 3-kinase/Akt extend the lifespan of the nematode *Caenorhabditis elegans*.¹⁷⁶ The forkhead transcription factor, Daf-16, which is phosphorylated and inactivated by Akt, plays an essential role in this longevity pathway. Recently, it was reported that the genes regulating longevity are conserved in organisms ranging from yeast to mice (Figure 2).^{177–180} These mutations that extend the lifespan are associated with an increase in resistance to oxidative stress, which is partly mediated by the increased expression of antioxidant genes.^{181–185}

A more recent study demonstrated a critical role of the insulin/Akt signaling pathway in regulating the lifespan of primary cultured human endothelial cells in a similar manner to its control of the longevity of *C elegans* (Figure 2).¹⁸⁶

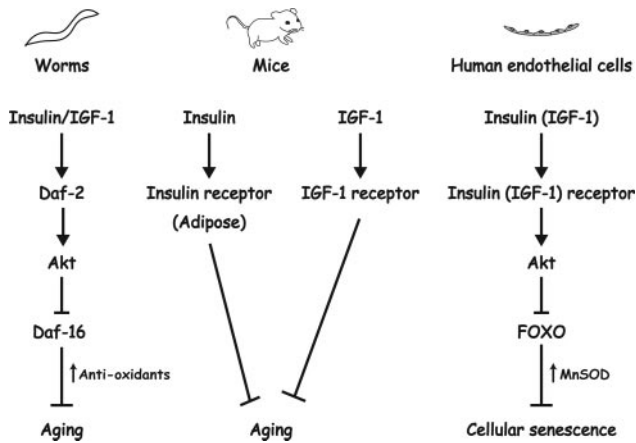


Figure 2. Conserved regulation of longevity. Reduced insulin/insulin-like growth factor-1 (IGF-1) signals increase the longevity in various species such as worms and mice. In worm, activation of the forkhead transcription factor Daf-16 is essential for lifespan extension. Moreover, induction of antioxidant genes by Daf-16 is required for longevity extension. Insulin-like growth factor-1 receptor heterozygous mice as well as mice lacking the insulin receptor in adipose tissues live longer. However, whether FOXO proteins are responsible for lifespan extension is unknown. Insulin/Akt signals negatively regulate the lifespan of human endothelial cells. Induction of antioxidant genes such as manganese superoxide dismutase (MnSOD) by FOXO is crucial for the regulation of endothelial lifespan by the insulin/Akt signals.

Treatment with high doses of insulin caused an increase of p53 activity and p21 expression, thereby accelerating the senescence of human endothelial cells. Activation of Akt by insulin leads to the phosphorylation of forkhead transcription factor (FOXO), a mammalian homolog of Daf-16, and thereby inhibits the transcription of antioxidant genes such as manganese superoxide dismutase. FOXO influences p53 activity by regulating levels of ROS.

What are the implications of these findings for human disease? Because various growth factors that contribute to atherosclerosis have been shown to increase Akt activity,¹⁸⁷ atherogenic stimuli may activate Akt in the vasculature and thus promote atherogenesis. Consistent with this notion, activation of Akt has been observed in human atheroma specimens, but not in normal arteries.¹⁸⁶ Constitutive activation of Akt in human vascular endothelial cells not only leads to cellular senescence but also to vascular dysfunction, such as impairment of angiogenesis and an increase of inflammation.¹⁸⁶ Insulin resistance and hyperinsulinemia are essential features of type 2 diabetes that may induce Akt-dependent vascular cell senescence. However, previous studies have suggested that activation of Akt, but not extracellular signal-regulated kinase, by insulin is significantly impaired in the vessels of diabetic patients and that such pathway-selective insulin resistance may induce macrovascular complications.¹⁸⁸ In contrast, some recent studies have shown that the basal level of tissue Akt activity is higher in diabetic patients than in normal subjects, although there is no significant difference in the response of Akt to insulin.¹⁸⁹ Constitutive activation of Akt is observed in the vessel walls of diabetic animals with hyperinsulinemia, but Akt activity is markedly reduced in the target organs of insulin such as adipose

tissue.¹⁸⁶ Such tissue-selective insulin resistance induces systemic hyperinsulinemia and leads to Akt-dependent vascular cell senescence, which may have a role in the vascular complications of diabetes.

Rosso et al¹⁹⁰ demonstrated that EPCs from diabetic donors undergo premature senescence and that activation of the Akt signaling pathway is essential for such senescence to occur. Brodsky et al¹⁹¹ reported that endothelial cell senescence also occurs in the arteries of type 2 diabetic rats and that expression of cell cycle proteins (such as p53 and p16) is induced in the endothelium of these diabetic animals. Moreover, these animals display evidence of vascular dysfunction, such as impaired endothelium-dependent relaxation and reduced angiogenesis, whereas treatment of these rats with antioxidants prevents endothelial senescence and ameliorates the above changes. Yokoi et al recently demonstrated that high glucose also induces endothelial cell senescence.¹⁹² Taken together, these findings suggest that diabetes promotes the senescence of EPCs as well as endothelial cells via the insulin/Akt pathway and/or the high glucose-induced signaling pathway, resulting in the development of diabetic vascular complications. Increased plasma and tissue levels of proinflammatory cytokines and prothrombotic factors have been demonstrated to exacerbate insulin resistance and to contribute to diabetic complications,^{193,194} and both of these changes are well-known features of cells undergoing senescence *in vitro*.⁴ Accordingly, type 2 diabetes can be regarded as a premature aging syndrome in which the dysregulation of insulin/Akt signaling promotes cellular senescence and leads to various complications. This suggests that antisenesence therapy might be effective for the treatment of not only diabetic vasculopathy but also other complications and insulin resistance.

Conclusion

There is evidence for a critical role of cellular senescence in the process of aging and various age-related diseases, including atherosclerosis. Antisenesence therapy is now emerging as a novel strategy for the treatment of human atherosclerosis, and several target molecules could be considered. One candidate is telomerase. There have been a number of reports demonstrating that telomerase is activated by medications or humoral factors that are known to exert a beneficial effect on cardiovascular disease, such as statins,¹⁹⁵ thiazolidinediones,¹⁹⁶ aspirin,¹³⁸ and estrogen.¹⁹⁷ Ang II type 1 receptor antagonists may also be useful for the treatment of vascular aging by suppressing Ang II-induced senescence.⁷⁴ p53 antagonists are available,¹⁹⁸ but systemic inhibition of p53 activity may induce tumorigenesis. It has also been reported that calorie restriction extends the lifespan of yeasts by activating the nicotinamide-adenine dinucleotide-dependent histone deacetylase Sir2.¹⁹⁹ Increased activity of sirtuins, its functional ortholog, has been shown to extend the lifespan of mammalian cells.²⁰⁰ Thus, calorie restriction mimetics including sirtuin activators²⁰¹ may be among of the best candidates for antisenesence therapy. Tissue-specific inhibition of the signaling pathways for senescence also increases antisenesence signals in a non-cell autonomous manner^{177,179,180,202} and has the potential to ameliorate age-

associated diseases such as atherosclerosis. Thus, the factors that mediate this intriguing mechanism may be another candidate for antisenesescence therapy. Identification of such factors will help to provide new methods for the treatment of human atherosclerosis.

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References

- Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises. Part I: Aging arteries: a "set up" for vascular disease. *Circulation*. 2003;107:139–146.
- Braunwald E, Zipes DP. *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine*. 7th ed. Philadelphia, Pa; London: Saunders; 2005.
- Faragher RG, Kipling D. How might replicative senescence contribute to human ageing? *Bioessays*. 1998;20:985–991.
- Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell*. 2005;120:513–522.
- Thompson KV, Holliday R. Genetic effects on the longevity of cultured human fibroblasts. II. DNA repair deficient syndromes. *Gerontology*. 1983;29:83–88.
- Martin GM. Genetic modulation of senescent phenotypes in Homo sapiens. *Cell*. 2005;120:523–532.
- Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res*. 1961;25:585–621.
- Hayflick L. The limited in vitro lifetime of human diploid cell strains. *Exp Cell Res*. 1965;37:614–636.
- Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352:1685–1695.
- Marin J. Age-related changes in vascular responses: a review. *Mech Ageing Dev*. 1995;79:71–114.
- Rivard A, Fabre JE, Silver M, Chen D, Murohara T, Kearney M, Magner M, Asahara T, Isner JM. Age-dependent impairment of angiogenesis. *Circulation*. 1999;99:111–120.
- Rivard A, Berthou-Soulie L, Principe N, Kearney M, Curry C, Branellec D, Semenza GL, Isner JM. Age-dependent defect in vascular endothelial growth factor expression is associated with reduced hypoxia-inducible factor 1 activity. *J Biol Chem*. 2000;275:29643–29647.
- Reed MJ, Edelberg JM. Impaired angiogenesis in the aged. *Sci Aging Knowledge Environ*. 2004;2004:pe7.
- Schneiderman J, Sawdey MS, Keeton MR, Bordin GM, Bernstein EF, Dilley RB, Loskutov DJ. Increased type 1 plasminogen activator inhibitor gene expression in atherosclerotic human arteries. *Proc Natl Acad Sci U S A*. 1992;89:6998–7002.
- Wilkerson WR, Sane DC. Aging and thrombosis. *Semin Thromb Hemost*. 2002;28:555–568.
- Lakatta EG. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises. Part III: Cellular and molecular clues to heart and arterial aging. *Circulation*. 2003;107:490–497.
- Sato I, Morita I, Kaji K, Ikeda M, Nagao M, Murota S. Reduction of nitric oxide producing activity associated with in vitro aging in cultured human umbilical vein endothelial cell. *Biochem Biophys Res Commun*. 1993;195:1070–1076.
- Matsushita H, Chang E, Glassford AJ, Cooke JP, Chiu CP, Tsao PS. eNOS activity is reduced in senescent human endothelial cells: preservation by hTERT immortalization. *Circ Res*. 2001;89:793–798.
- Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation*. 2002;105:1541–1544.
- Xin MG, Zhang J, Block ER, Patel JM. Senescence-enhanced oxidative stress is associated with deficiency of mitochondrial cytochrome c oxidase in vascular endothelial cells. *Mech Ageing Dev*. 2003;124:911–919.
- Macip S, Igarashi M, Fang L, Chen A, Pan ZQ, Lee SW, Aaronson SA. Inhibition of p21-mediated ROS accumulation can rescue p21-induced senescence. *EMBO J*. 2002;21:2180–2188.
- Lee AC, Fenster BE, Ito H, Takeda K, Bae NS, Hirai T, Yu ZX, Ferrans VJ, Howard BH, Finkel T. Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. *J Biol Chem*. 1999;274:7936–7940.
- Haendeler J, Hoffmann J, Diehl JF, Vasa M, Spyridopoulos I, Zeiher AM, Dimmeler S. Antioxidants inhibit nuclear export of telomerase reverse transcriptase and delay replicative senescence of endothelial cells. *Circ Res*. 2004;94:768–775.
- Deshpande SS, Qi B, Park YC, Irani K. Constitutive activation of rac1 results in mitochondrial oxidative stress and induces premature endothelial cell senescence. *Arterioscler Thromb Vasc Biol*. 2003;23:e1–e6.
- Unterluggauer H, Hampel B, Zwierschke W, Jansen-Durr P. Senescence-associated cell death of human endothelial cells: the role of oxidative stress. *Exp Gerontol*. 2003;38:1149–1160.
- van der Loo B, Labugger R, Skepper JN, Bachschmid M, Kilo J, Powell JM, Palacios-Callender M, Erusalimsky JD, Quaschnig T, Malinski T, Gygi D, Ullrich V, Luscher TF. Enhanced peroxynitrite formation is associated with vascular aging. *J Exp Med*. 2000;192:1731–1744.
- Nakajima M, Hashimoto M, Wang F, Yamanaga K, Nakamura N, Uchida T, Yamanouchi K. Aging decreases the production of PGI2 in rat aortic endothelial cells. *Exp Gerontol*. 1997;32:685–693.
- Sato I, Kaji K, Morita I, Nagao M, Murota S. Augmentation of endothelin-1, prostacyclin and thromboxane A2 secretion associated with in vitro ageing in cultured human umbilical vein endothelial cells. *Mech Ageing Dev*. 1993;71:73–84.
- Neubert K, Haberland A, Kruse I, Wirth M, Schimke I. The ratio of formation of prostacyclin/thromboxane A2 in HUVEC decreased in each subsequent passage. *Prostaglandins*. 1997;54:447–462.
- Comi P, Chiamonte R, Maier JA. Senescence-dependent regulation of type 1 plasminogen activator inhibitor in human vascular endothelial cells. *Exp Cell Res*. 1995;219:304–308.
- West MD, Shay JW, Wright WE, Linskens MH. Altered expression of plasminogen activator and plasminogen activator inhibitor during cellular senescence. *Exp Gerontol*. 1996;31:175–193.
- Garfinkel S, Brown S, Wessendorf JH, Maciag T. Post-transcriptional regulation of interleukin 1 alpha in various strains of young and senescent human umbilical vein endothelial cells. *Proc Natl Acad Sci U S A*. 1994;91:1559–1563.
- Maier JA, Statuto M, Ragnotti G. Senescence stimulates U937-endothelial cell interactions. *Exp Cell Res*. 1993;208:270–274.
- Shelton DN, Chang E, Whittier PS, Choi D, Funk WD. Microarray analysis of replicative senescence. *Curr Biol*. 1999;9:939–945.
- Maier JA, Voulalas P, Roeder D, Maciag T. Extension of the life-span of human endothelial cells by an interleukin-1 alpha antisense oligomer. *Science*. 1990;249:1570–1574.
- Yang J, Chang E, Cherry AM, Bangs CD, Oei Y, Bodnar A, Bronstein A, Chiu CP, Herron GS. Human endothelial cell life extension by telomerase expression. *J Biol Chem*. 1999;274:26141–26148.
- Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997;275:964–967.
- Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med*. 1999;5:434–438.
- Werner N, Nickenig G. Clinical and therapeutic implications of EPC biology in atherosclerosis. *J Cell Mol Med*. 2006;10:318–332.
- Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med*. 2003;348:593–600.
- Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, Zeiher AM, Dimmeler S. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res*. 2001;89:e1–e7.
- Crass MF 3rd, Borst SE, Scarpace PJ. Beta-adrenergic responsiveness in cultured aorta smooth muscle cells. Effects of subculture and aging. *Biochem Pharmacol*. 1992;43:1811–1815.
- Robert L, Robert AM, Jacotot B. Elastin-elastase-atherosclerosis revisited. *Atherosclerosis*. 1998;140:281–295.
- Kumazaki T, Kobayashi M, Mitsui Y. Enhanced expression of fibronectin during in vivo cellular aging of human vascular endothelial cells and skin fibroblasts. *Exp Cell Res*. 1993;205:396–402.

45. Kumazaki T, Robetorye RS, Robetorye SC, Smith JR. Fibronectin expression increases during in vitro cellular senescence: correlation with increased cell area. *Exp Cell Res*. 1991;195:13–19.
46. Wang E. Senescent human fibroblasts resist programmed cell death, and failure to suppress bcl2 is involved. *Cancer Res*. 1995;55:2284–2292.
47. Hampel B, Malisan F, Niederegger H, Testi R, Jansen-Durr P. Differential regulation of apoptotic cell death in senescent human cells. *Exp Gerontol*. 2004;39:1713–1721.
48. Zhang J, Patel JM, Block ER. Enhanced apoptosis in prolonged cultures of senescent porcine pulmonary artery endothelial cells. *Mech Ageing Dev*. 2002;123:613–625.
49. Mallat Z, Tedgui A. Current perspective on the role of apoptosis in atherothrombotic disease. *Circ Res*. 2001;88:998–1003.
50. Kario K, Pickering TG, Matsuo T, Hoshida S, Schwartz JE, Shimada K. Stroke prognosis and abnormal nocturnal blood pressure falls in older hypertensives. *Hypertension*. 2001;38:852–857.
51. Verdecchia P, Porcellati C, Schillaci G, Borgioni C, Ciucci A, Battistelli M, Guerrieri M, Gatteschi C, Zampi I, Santucci A, Santucci C, Reboldi G. Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension. *Hypertension*. 1994;24:793–801.
52. Kunieda T, Minamino T, Katsuno T, Tateno K, Nishi J, Miyauchi H, Orimo M, Okada S, Komuro I. Cellular senescence impairs circadian expression of clock genes in vitro and in vivo. *Circ Res*. 2006;98:532–539.
53. Bennett MR, Macdonald K, Chan SW, Boyle JJ, Weissberg PL. Cooperative interactions between RB and p53 regulate cell proliferation, cell senescence, and apoptosis in human vascular smooth muscle cells from atherosclerotic plaques. *Circ Res*. 1998;82:704–712.
54. Liao S, Curci JA, Kelley BJ, Sicard GA, Thompson RW. Accelerated replicative senescence of medial smooth muscle cells derived from abdominal aortic aneurysms compared to the adjacent inferior mesenteric artery. *J Surg Res*. 2000;92:85–95.
55. Buring KF. The endothelium of advanced arteriosclerotic plaques in humans. *Arterioscler Thromb*. 1991;11:1678–1689.
56. Ross R, Wight TN, Strandness E, Thiele B. Human atherosclerosis. I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery. *Am J Pathol*. 1984;114:79–93.
57. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, Peacocke M, Campisi J. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A*. 1995;92:9363–9367.
58. Fenton M, Barker S, Kurz DJ, Erusalimsky JD. Cellular senescence after single and repeated balloon catheter denudations of rabbit carotid arteries. *Arterioscler Thromb Vasc Biol*. 2001;21:220–226.
59. Vasile E, Tomita Y, Brown LF, Kocher O, Dvorak HF. Differential expression of thymosin beta-10 by early passage and senescent vascular endothelium is modulated by VPF/VEGF: evidence for senescent endothelial cells in vivo at sites of atherosclerosis. *FASEB J*. 2001;15:458–466.
60. Minamino T, Yoshida T, Tateno K, Miyauchi H, Zou Y, Toko H, Komuro I. Ras induces vascular smooth muscle cell senescence and inflammation in human atherosclerosis. *Circulation*. 2003;108:2264–2269.
61. Campisi J, Kim SH, Lim CS, Rubio M. Cellular senescence, cancer and aging: the telomere connection. *Exp Gerontol*. 2001;36:1619–1637.
62. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. *Science*. 1994;266:2011–2015.
63. Morrison SJ, Prowse KR, Ho P, Weissman IL. Telomerase activity in hematopoietic cells is associated with self-renewal potential. *Immunity*. 1996;5:207–216.
64. Lee HW, Blasco MA, Gottlieb GJ, Horner JW 2nd, Greider CW, DePinho RA. Essential role of mouse telomerase in highly proliferative organs. *Nature*. 1998;392:569–574.
65. Smith LL, Collier HA, Roberts JM. Telomerase modulates expression of growth-controlling genes and enhances cell proliferation. *Nat Cell Biol*. 2003;5:474–479.
66. Masutomi K, Yu EY, Khurts S, Ben-Porath I, Currier JL, Metz GB, Brooks MW, Kaneko S, Murakami S, DeCaprio JA, Weinberg RA, Stewart SA, Hahn WC. Telomerase maintains telomere structure in normal human cells. *Cell*. 2003;114:241–253.
67. Minamino T, Kourembanas S. Mechanisms of telomerase induction during vascular smooth muscle cell proliferation. *Circ Res*. 2001;89:237–243.
68. Minamino T, Mitsialis SA, Kourembanas S. Hypoxia extends the life span of vascular smooth muscle cells through telomerase activation. *Mol Cell Biol*. 2001;21:3336–3342.
69. Hsiao R, Sharma HW, Ramakrishnan S, Keith E, Narayanan R. Telomerase activity in normal human endothelial cells. *Anticancer Res*. 1997;17:827–832.
70. Chang E, Harley CB. Telomere length and replicative aging in human vascular tissues. *Proc Natl Acad Sci U S A*. 1995;92:11190–11194.
71. Serrano M, Blasco MA. Putting the stress on senescence. *Curr Opin Cell Biol*. 2001;13:748–753.
72. Lombard DB, Chua KF, Mostoslavsky R, Franco S, Gostissa M, Alt FW. DNA repair, genome stability, and aging. *Cell*. 2005;120:497–512.
73. Bandyopadhyay D, Medrano EE. The emerging role of epigenetics in cellular and organismal aging. *Exp Gerontol*. 2003;38:1299–1307.
74. Kunieda T, Minamino T, Nishi J, Tateno K, Oyama T, Katsuno T, Miyauchi H, Orimo M, Okada S, Takamura M, Nagai T, Kaneko S, Komuro I. Angiotensin II induces premature senescence of vascular smooth muscle cells and accelerates the development of atherosclerosis via a p21-dependent pathway. *Circulation*. 2006;114:953–960.
75. Matthews C, Gorenne I, Scott S, Figg N, Kirkpatrick P, Ritchie A, Goddard M, Bennett M. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis: effects of telomerase and oxidative stress. *Circ Res*. 2006;99:156–164.
76. Campisi J. Cellular senescence as a tumor-suppressor mechanism. *Trends Cell Biol*. 2001;11:S27–S31.
77. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. *Cancer Cell*. 2002;2:103–112.
78. Wahl GM, Carr AM. The evolution of diverse biological responses to DNA damage: insights from yeast and p53. *Nat Cell Biol*. 2001;3:E277–E286.
79. Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol Cell*. 2004;14:501–513.
80. d'Adda di Fagnana F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP. A DNA damage checkpoint response in telomere-initiated senescence. *Nature*. 2003;426:194–198.
81. Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Curr Biol*. 2003;13:1549–1556.
82. Herbig U, Ferreira M, Condel L, Carey D, Sedivy JM. Cellular senescence in aging primates. *Science*. 2006;311:1257.
83. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell*. 1997;88:593–602.
84. Ferbeyre G, de Stanchina E, Querido E, Baptiste N, Prives C, Lowe SW. PML is induced by oncogenic ras and promotes premature senescence. *Genes Dev*. 2000;14:2015–2027.
85. Pearson M, Carbone R, Sebastiani C, Cioce M, Fagioli M, Saito S, Higashimoto Y, Appella E, Minucci S, Pandolfi PP, Pelicci PG. PML regulates p53 acetylation and premature senescence induced by oncogenic Ras. *Nature*. 2000;406:207–210.
86. Lowe SW, Sherr CJ. Tumor suppression by Ink4a-Arf: progress and puzzles. *Curr Opin Genet Dev*. 2003;13:77–83.
87. Holst CR, Nuovo GJ, Esteller M, Chew K, Baylin SB, Herman JG, Tlsty TD. Methylation of p16(INK4a) promoters occurs in vivo in histologically normal human mammary epithelia. *Cancer Res*. 2003;63:1596–1601.
88. Itahana K, Zou Y, Itahana Y, Martinez JL, Beausejour C, Jacobs JJ, Van Lohuizen M, Band V, Campisi J, Dimri GP. Control of the replicative life span of human fibroblasts by p16 and the polycomb protein Bmi-1. *Mol Cell Biol*. 2003;23:389–401.
89. Watanabe Y, Lee SW, Detmar M, Ajioka I, Dvorak HF. Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) delays and induces escape from senescence in human dermal microvascular endothelial cells. *Oncogene*. 1997;14:2025–2032.
90. Sharpless NE, Bardeesy N, Lee KH, Carrasco D, Castrillon DH, Aguirre AJ, Wu EA, Horner JW, DePinho RA. Loss of p16INK4a with retention of p19Arf predisposes mice to tumorigenesis. *Nature*. 2001;413:86–91.
91. Beausejour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, Campisi J. Reversal of human cellular senescence: roles of the p53 and p16 pathways. *EMBO J*. 2003;22:4212–4222.
92. Narita M, Narita M, Krizhanovsky V, Nunez S, Chicas A, Hearn SA, Myers MP, Lowe SW. A novel role for high-mobility group A proteins

- in cellular senescence and heterochromatin formation. *Cell*. 2006;126:503–514.
93. Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell*. 2003;113:703–716.
 94. Zhang R, Poustovoitov MV, Ye X, Santos HA, Chen W, Daganzo SM, Erzberger JP, Serebriiskii IG, Canutescu AA, Dunbrack RL, Pehrson JR, Berger JM, Kaufman PD, Adams PD. Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. *Dev Cell*. 2005;8:19–30.
 95. Aviv H, Khan MY, Skurnick J, Okuda K, Kimura M, Gardner J, Priolo L, Aviv A. Age dependent aneuploidy and telomere length of the human vascular endothelium. *Atherosclerosis*. 2001;159:281–287.
 96. Ogami M, Ikura Y, Ohsawa M, Matsuo T, Kayo S, Yoshimi N, Hai E, Shirai N, Ehara S, Komatsu R, Naruko T, Ueda M. Telomere shortening in human coronary artery diseases. *Arterioscler Thromb Vasc Biol*. 2004;24:546–550.
 97. Benetos A, Okuda K, Lajemi M, Kimura M, Thomas F, Skurnick J, Labat C, Bean K, Aviv A. Telomere length as an indicator of biological aging: the gender effect and relation with pulse pressure and pulse wave velocity. *Hypertension*. 2001;37:381–385.
 98. Samani NJ, Boulthby R, Butler R, Thompson JR, Goodall AH. Telomere shortening in atherosclerosis. *Lancet*. 2001;358:472–473.
 99. Brouillette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. *Arterioscler Thromb Vasc Biol*. 2003;23:842–846.
 100. Benetos A, Gardner JP, Zureik M, Labat C, Xiaobin L, Adamopoulos C, Temmar M, Bean KE, Thomas F, Aviv A. Short telomeres are associated with increased carotid atherosclerosis in hypertensive subjects. *Hypertension*. 2004;43:182–185.
 101. Kurz DJ, Kloeckener-Gruissem B, Akhmedov A, Eberli FR, Buhler I, Berger W, Bertel O, Luscher TF. Degenerative aortic valve stenosis, but not coronary disease, is associated with shorter telomere length in the elderly. *Arterioscler Thromb Vasc Biol*. 2006;26:e114–e117.
 102. von Zglinicki T, Serra V, Lorenz M, Saretzki G, Lenzen-Grossimlghaus R, Gessner R, Risch A, Steinhagen-Thiessen E. Short telomeres in patients with vascular dementia: an indicator of low antioxidative capacity and a possible risk factor? *Lab Invest*. 2000;80:1739–1747.
 103. Jeanclous E, Krolewski A, Skurnick J, Kimura M, Aviv H, Warram JH, Aviv A. Shortened telomere length in white blood cells of patients with IDDM. *Diabetes*. 1998;47:482–486.
 104. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD, Cawthon RM. Accelerated telomere shortening in response to life stress. *Proc Natl Acad Sci U S A*. 2004;101:17312–17315.
 105. Gardner JP, Li S, Srinivasan SR, Chen W, Kimura M, Lu X, Berenson GS, Aviv A. Rise in insulin resistance is associated with escalated telomere attrition. *Circulation*. 2005;111:2171–2177.
 106. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, Aviv A, Spector TD. Obesity, cigarette smoking, and telomere length in women. *Lancet*. 2005;366:662–664.
 107. Demissie S, Levy D, Benjamin EJ, Cupples LA, Gardner JP, Herbert A, Kimura M, Larson MG, Meigs JB, Keaney JF, Aviv A. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. *Aging Cell*. 2006;5:325–330.
 108. Epel ES, Lin J, Wilhelm FH, Wolkowitz OM, Cawthon R, Adler NE, Dolbier C, Mendes WB, Blackburn EH. Cell aging in relation to stress arousal and cardiovascular disease risk factors. *Psychoneuroendocrinology*. 2006;31:277–287.
 109. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet*. 2003;361:393–395.
 110. Bischoff C, Petersen HC, Graakjaer J, Andersen-Ranberg K, Vaupel JW, Bohr VA, Kolvraa S, Christensen K. No association between telomere length and survival among the elderly and oldest old. *Epidemiology*. 2006;17:190–194.
 111. Martin-Ruiz CM, Gussekloo J, van Heemst D, von Zglinicki T, Westendorp RG. Telomere length in white blood cells is not associated with morbidity or mortality in the oldest old: a population-based study. *Aging Cell*. 2005;4:287–290.
 112. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, Greider CW. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell*. 1997;91:25–34.
 113. Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C, DePinho RA. Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell*. 1999;96:701–712.
 114. Franco S, Segura I, Riese HH, Blasco MA. Decreased B16F10 melanoma growth and impaired vascularization in telomerase-deficient mice with critically short telomeres. *Cancer Res*. 2002;62:552–559.
 115. Poch E, Carbonell P, Franco S, Diez-Juan A, Blasco MA, Andres V. Short telomeres protect from diet-induced atherosclerosis in apolipoprotein E-null mice. *FASEB J*. 2004;18:418–420.
 116. Perez-Rivero G, Ruiz-Torres MP, Rivas-Elena JV, Jerkic M, Diez-Marques ML, Lopez-Novoa JM, Blasco MA, Rodriguez-Puyol D. Mice deficient in telomerase activity develop hypertension because of an excess of endothelin production. *Circulation*. 2006;114:309–317.
 117. Cao Y, Li H, Mu FT, Ebisui O, Funder JW, Liu JP. Telomerase activation causes vascular smooth muscle cell proliferation in genetic hypertension. *FASEB J*. 2002;16:96–98.
 118. Minamino T, Komuro I. The role of telomerase activation in the regulation of vascular smooth muscle cell proliferation. *Drug News Perspect*. 2003;16:211–216.
 119. Najjar SS, Scuteri A, Lakatta EG. Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension*. 2005;46:454–462.
 120. Merched AJ, Chan L. Absence of p21Waf1/Cip1/Sdi1 modulates macrophage differentiation and inflammatory response and protects against atherosclerosis. *Circulation*. 2004;110:3830–3841.
 121. Ihling C, Menzel G, Wellens E, Monting JS, Schaefer HE, Zeiher AM. Topographical association between the cyclin-dependent kinases inhibitor P21, p53 accumulation, and cellular proliferation in human atherosclerotic tissue. *Arterioscler Thromb Vasc Biol*. 1997;17:2218–2224.
 122. Guevara NV, Kim HS, Antonova EI, Chan L. The absence of p53 accelerates atherosclerosis by increasing cell proliferation in vivo. *Nat Med*. 1999;5:335–339.
 123. van Vlijmen BJ, Gerritsen G, Franken AL, Boesten LS, Kockx MM, Gijbels MJ, Vierboom MP, van Eck M, van De Water B, van Berkel TJ, Havekes LM. Macrophage p53 deficiency leads to enhanced atherosclerosis in APOE*3-Leiden transgenic mice. *Circ Res*. 2001;88:780–786.
 124. von der Thusen JH, van Vlijmen BJ, Hoeben RC, Kockx MM, Havekes LM, van Berkel TJ, Biessen EA. Induction of atherosclerotic plaque rupture in apolipoprotein E-/- mice after adenovirus-mediated transfer of p53. *Circulation*. 2002;105:2064–2070.
 125. George J, Afek A, Keren P, Herz I, Goldberg I, Haklai R, Kloog Y, Keren G. Functional inhibition of Ras by S-trans,trans-farnesyl thio-salicylic acid attenuates atherosclerosis in apolipoprotein E knockout mice. *Circulation*. 2002;105:2416–2422.
 126. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature*. 2000;408:239–247.
 127. Kurz DJ, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky JD. Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *J Cell Sci*. 2004;117:2417–2426.
 128. Xu D, Neville R, Finkel T. Homocysteine accelerates endothelial cell senescence. *FEBS Lett*. 2000;470:20–24.
 129. Breitschopf K, Zeiher AM, Dimmeler S. Pro-atherogenic factors induce telomerase inactivation in endothelial cells through an Akt-dependent mechanism. *FEBS Lett*. 2001;493:21–25.
 130. Maier JA, Barengli L, Bradamante S, Pagani F. Induction of human endothelial cell growth by mildly oxidized low density lipoprotein. *Atherosclerosis*. 1996;123:115–121.
 131. Furumoto K, Inoue E, Nagao N, Hiyaama E, Miwa N. Age-dependent telomere shortening is slowed down by enrichment of intracellular vitamin C via suppression of oxidative stress. *Life Sci*. 1998;63:935–948.
 132. Vasa M, Breitschopf K, Zeiher AM, Dimmeler S. Nitric oxide activates telomerase and delays endothelial cell senescence. *Circ Res*. 2000;87:540–542.
 133. Venable ME, Webb-Froehlich LM, Sloan EF, Thomley JE. Shift in sphingolipid metabolism leads to an accumulation of ceramide in senescence. *Mech Ageing Dev*. 2006;127:473–480.
 134. Zhang J, Block ER, Patel JM. Down-regulation of mitochondrial cytochrome c oxidase in senescent porcine pulmonary artery endothelial cells. *Mech Ageing Dev*. 2002;123:1363–1374.
 135. Kumazaki T, Sakano T, Yoshida T, Hamada K, Sumida H, Teranishi Y, Nishiyama M, Mitsui Y. Enhanced expression of mitochondrial genes in senescent endothelial cells and fibroblasts. *Mech Ageing Dev*. 1998;101:91–99.

136. Chen J, Brodsky SV, Goligorsky DM, Hampel DJ, Li H, Gross SS, Goligorsky MS. Glycated collagen I induces premature senescence-like phenotypic changes in endothelial cells. *Circ Res*. 2002;90:1290–1298.
137. Scalera F, Borlak J, Beckmann B, Martens-Lobenhoffer J, Thum T, Tager M, Bode-Boger SM. Endogenous nitric oxide synthesis inhibitor asymmetric dimethyl L-arginine accelerates endothelial cell senescence. *Arterioscler Thromb Vasc Biol*. 2004;24:1816–1822.
138. Bode-Boger SM, Martens-Lobenhoffer J, Tager M, Schroder H, Scalera F. Aspirin reduces endothelial cell senescence. *Biochem Biophys Res Commun*. 2005;334:1226–1232.
139. Scalera F, Martens-Lobenhoffer J, Tager M, Bukowska A, Lendeckel U, Bode-Boger SM. Effect of L-arginine on asymmetric dimethylarginine (ADMA) or homocysteine-accelerated endothelial cell aging. *Biochem Biophys Res Commun*. 2006;345:1075–1082.
140. Oh CW, Bump EA, Kim JS, Janigro D, Mayberg MR. Induction of a senescence-like phenotype in bovine aortic endothelial cells by ionizing radiation. *Radiat Res*. 2001;156:232–240.
141. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. *Cell*. 2005;120:483–495.
142. Passos JF, von Zglinicki T. Mitochondria, telomeres and cell senescence. *Exp Gerontol*. 2005;40:466–472.
143. Puddu P, Puddu GM, Galletti L, Cravero E, Muscare A. Mitochondrial dysfunction as an initiating event in atherogenesis: a plausible hypothesis. *Cardiology*. 2005;103:137–141.
144. Ballinger SW, Patterson C, Knight-Lozano CA, Burow DL, Conklin CA, Hu Z, Reuf J, Horaist C, Lebovitz R, Hunter GC, McIntyre K, Runge MS. Mitochondrial integrity and function in atherogenesis. *Circulation*. 2002;106:544–549.
145. Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly YM, Gidlof S, Oldfors A, Wibom R, Tornell J, Jacobs HT, Larsson NG. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature*. 2004;429:417–423.
146. Schriener SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, Rabinovitch PS. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science*. 2005;308:1909–1911.
147. Trifunovic A, Hansson A, Wredenberg A, Rovio AT, Dufour E, Khvorostov I, Spelbrink JN, Wibom R, Jacobs HT, Larsson NG. Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. *Proc Natl Acad Sci U S A*. 2005;102:17993–17998.
148. Kujto GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamasoba T, Tanokura M, Weindrich R, Leeuwenburgh C, Prolla TA. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science*. 2005;309:481–484.
149. Hasty P, Campisi J, Hoeijmakers J, van Steeg H, Vijg J. Aging and genome maintenance: lessons from the mouse? *Science*. 2003;299:1355–1359.
150. Wong KK, Maser RS, Bachoo RM, Menon J, Carrasco DR, Gu Y, Alt FW, DePinho RA. Telomere dysfunction and Atm deficiency compromises organ homeostasis and accelerates ageing. *Nature*. 2003;421:643–648.
151. de Boer J, Andressoo JO, de Wit J, Huijman J, Beems RB, van Steeg H, Weeda G, van der Horst GT, van Leeuwen W, Themmen AP, Meradji M, Hoeijmakers JH. Premature aging in mice deficient in DNA repair and transcription. *Science*. 2002;296:1276–1279.
152. Vogel H, Lim DS, Karsenty G, Finegold M, Hasty P. Deletion of Ku86 causes early onset of senescence in mice. *Proc Natl Acad Sci U S A*. 1999;96:10770–10775.
153. Espejel S, Martin M, Klatt P, Martin-Caballero J, Flores JM, Blasco MA. Shorter telomeres, accelerated ageing and increased lymphoma in DNA-PKcs-deficient mice. *EMBO Rep*. 2004;5:503–509.
154. Espejel S, Klatt P, Menissier-de Murcia J, Martin-Caballero J, Flores JM, Taccioli G, de Murcia G, Blasco MA. Impact of telomerase ablation on organismal viability, aging, and tumorigenesis in mice lacking the DNA repair proteins PARP-1, Ku86, or DNA-PKcs. *J Cell Biol*. 2004;167:627–638.
155. Weeda G, Donker I, de Wit J, Morreau H, Janssens R, Vissers CJ, Nigg A, van Steeg H, Bootsma D, Hoeijmakers JH. Disruption of mouse ERCC1 results in a novel repair syndrome with growth failure, nuclear abnormalities and senescence. *Curr Biol*. 1997;7:427–439.
156. Tian M, Shinkura R, Shinkura N, Alt FW. Growth retardation, early death, and DNA repair defects in mice deficient for the nucleotide excision repair enzyme XPF. *Mol Cell Biol*. 2004;24:1200–1205.
157. McWhir J, Selfridge J, Harrison DJ, Squires S, Melton DW. Mice with DNA repair gene (ERCC-1) deficiency have elevated levels of p53, liver nuclear abnormalities and die before weaning. *Nat Genet*. 1993;5:217–224.
158. Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, Lu X, Soron G, Cooper B, Brayton C, Hee Park S, Thompson T, Karsenty G, Bradley A, Donehower LA. p53 mutant mice that display early ageing-associated phenotypes. *Nature*. 2002;415:45–53.
159. Maier B, Gluba W, Bernier B, Turner T, Mohammad K, Guise T, Sutherland A, Thorne M, Scrable H. Modulation of mammalian life span by the short isoform of p53. *Genes Dev*. 2004;18:306–319.
160. Garcia-Cao I, Garcia-Cao M, Martin-Caballero J, Criado LM, Klatt P, Flores JM, Weill JC, Blasco MA, Serrano M. “Super p53” mice exhibit enhanced DNA damage response, are tumor resistant and age normally. *EMBO J*. 2002;21:6225–6235.
161. Cao L, Li W, Kim S, Brodie SG, Deng CX. Senescence, aging, and malignant transformation mediated by p53 in mice lacking the Bcr1 full-length isoform. *Genes Dev*. 2003;17:201–213.
162. Krishnamurthy J, Torrice C, Ramsey MR, Kovalev GI, Al-Regaiey K, Su L, Sharpless NE. Ink4a/Arf expression is a biomarker of aging. *J Clin Invest*. 2004;114:1299–1307.
163. Janzen V, Forkert R, Fleming HE, Saito Y, Waring MT, Dombkowski DM, Cheng T, DePinho RA, Sharpless NE, Scadden DT. Stem-cell ageing modified by the cyclin-dependent kinase inhibitor p16INK4a. *Nature*. 2006;443:421–426.
164. Molofsky AV, Slutsky SG, Joseph NM, He S, Pardoll R, Krishnamurthy J, Sharpless NE, Morrison SJ. Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing. *Nature*. 2006;443:448–452.
165. Krishnamurthy J, Ramsey MR, Ligon KL, Torrice C, Koh A, Bonner-Weir S, Sharpless NE. p16INK4a induces an age-dependent decline in islet regenerative potential. *Nature*. 2006;443:453–457.
166. Lebel M, Leder P. A deletion within the murine Werner syndrome helicase induces sensitivity to inhibitors of topoisomerase and loss of cellular proliferative capacity. *Proc Natl Acad Sci U S A*. 1998;95:13097–13102.
167. Chang S, Multani AS, Cabrera NG, Naylor ML, Laud P, Lombard D, Pathak S, Guarente L, DePinho RA. Essential role of limiting telomeres in the pathogenesis of Werner syndrome. *Nat Genet*. 2004;36:877–882.
168. Du X, Shen J, Kugan N, Furth EE, Lombard DB, Cheung C, Pak S, Luo G, Pignolo RJ, DePinho RA, Guarente L, Johnson FB. Telomere shortening exposes functions for the mouse Werner and Bloom syndrome genes. *Mol Cell Biol*. 2004;24:8437–8446.
169. De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C, Amiel J, Boccaccio I, Lyonnet S, Stewart CL, Munnich A, Le Merrer M, Levy N. Lamin A truncation in Hutchinson-Gilford progeria. *Science*. 2003;300:2055.
170. Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, Erdos MR, Robbins CM, Moses TY, Berglund P, Dutra A, Pak E, Durkin S, Csoka AB, Boehnke M, Glover TW, Collins FS. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature*. 2003;423:293–298.
171. Goldman RD, Shumaker DK, Erdos MR, Eriksson M, Goldman AE, Gordon LB, Gruenbaum Y, Khuon S, Mendez M, Varga R, Collins FS. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. *Proc Natl Acad Sci U S A*. 2004;101:8963–8968.
172. Shumaker DK, Dechat T, Kohlmaier A, Adam SA, Bozovsky MR, Erdos MR, Eriksson M, Goldman AE, Khuon S, Collins FS, Jenuwein T, Goldman RD. Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. *Proc Natl Acad Sci U S A*. 2006;103:8703–8708.
173. Fong LG, Frost D, Meta M, Qiao X, Yang SH, Coffinier C, Young SG. A protein farnesyltransferase inhibitor ameliorates disease in a mouse model of progeria. *Science*. 2006;311:1621–1623.
174. Longo VD, Finch CE. Evolutionary medicine: from dwarf model systems to healthy centenarians? *Science*. 2003;299:1342–1346.
175. Koubova J, Guarente L. How does calorie restriction work? *Genes Dev*. 2003;17:313–321.
176. Kenyon C. The plasticity of aging: insights from long-lived mutants. *Cell*. 2005;120:449–460.
177. Blüher M, Kahn BB, Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science*. 2003;299:572–574.

178. Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloën A, Even PC, Cervera P, Le Bouc Y. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature*. 2003;421:182–187.
179. Hwangbo DS, Gershman B, Tu MP, Palmer M, Tatar M. Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature*. 2004;429:562–566.
180. Giannakou ME, Goss M, Junger MA, Hafen E, Leevers SJ, Partridge L. Long-lived Drosophila with overexpressed dFOXO in adult fat body. *Science*. 2004;305:361.
181. Fabrizio P, Liou LL, Moy VN, Diaspro A, SelverstoneValentine J, Gralla EB, Longo VD. SOD2 functions downstream of Sch9 to extend longevity in yeast. *Genetics*. 2003;163:35–46.
182. Honda Y, Honda S. The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. *FASEB J*. 1999;13:1385–1393.
183. Taub J, Lau JF, Ma C, Hahn JH, Hoque R, Rothblatt J, Chalfie M. A cytosolic catalase is needed to extend adult lifespan in *C. elegans* daf-C and clk-1 mutants. *Nature*. 1999;399:162–166.
184. Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, Kenyon C. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature*. 2003;424:277–283.
185. Oh SW, Mukhopadhyay A, Dixit BL, Raha T, Green MR, Tissenbaum HA. Identification of direct DAF-16 targets controlling longevity, metabolism and diapause by chromatin immunoprecipitation. *Nat Genet*. 2006;38:251–257.
186. Miyauchi H, Minamino T, Tateno K, Kunieda T, Toko H, Komuro I. Akt negatively regulates the in vitro lifespan of human endothelial cells via a p53/p21-dependent pathway. *EMBO J*. 2004;23:212–220.
187. Cantley LC. The phosphoinositide 3-kinase pathway. *Science*. 2002;296:1655–1657.
188. Jiang ZY, Lin YW, Clemont A, Feener EP, Hein KD, Igarashi M, Yamauchi T, White MF, King GL. Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats. *J Clin Invest*. 1999;104:447–457.
189. Hojlund K, Staehr P, Hansen BF, Green KA, Hardie DG, Richter EA, Beck-Nielsen H, Wojtaszewski JF. Increased phosphorylation of skeletal muscle glycogen synthase at NH2-terminal sites during physiological hyperinsulinemia in type 2 diabetes. *Diabetes*. 2003;52:1393–1402.
190. Rosso A, Balsamo A, Gambino R, Dentelli P, Falcioni R, Cassader M, Pegoraro L, Pagano G, Brizzi MF. p53 Mediates the accelerated onset of senescence of endothelial progenitor cells in diabetes. *J Biol Chem*. 2006;281:4339–4347.
191. Brodsky SV, Gealekman O, Chen J, Zhang F, Togashi N, Crabtree M, Gross SS, Nasjletti A, Goligorsky MS. Prevention and reversal of premature endothelial cell senescence and vasculopathy in obesity-induced diabetes by ebselen. *Circ Res*. 2004;94:377–384.
192. Yokoi T, Fukuo K, Yasuda O, Hotta M, Miyazaki J, Takemura Y, Kawamoto H, Ichijo H, Ogihara T. Apoptosis signal-regulating kinase 1 mediates cellular senescence induced by high glucose in endothelial cells. *Diabetes*. 2006;55:1660–1665.
193. Rui L, Aguirre V, Kim JK, Shulman GI, Lee A, Corbould A, Dunaif A, White MF. Insulin/IGF-1 and TNF-alpha stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. *J Clin Invest*. 2001;107:181–189.
194. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, Yamashita S, Miura M, Fukuda Y, Takemura K, Tokunaga K, Matsuzawa Y. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med*. 1996;2:800–803.
195. Assmus B, Urbich C, Aicher A, Hofmann WK, Haendeler J, Rossig L, Spyridopoulos I, Zeiher AM, Dimmeler S. HMG-CoA reductase inhibitors reduce senescence and increase proliferation of endothelial progenitor cells via regulation of cell cycle regulatory genes. *Circ Res*. 2003;92:1049–1055.
196. Ogawa D, Nomiya T, Nakamachi T, Heywood EB, Stone JF, Berger JP, Law RE, Brummer D. Activation of peroxisome proliferator-activated receptor gamma suppresses telomerase activity in vascular smooth muscle cells. *Circ Res*. 2006;98:e50–e59.
197. Misiti S, Nanni S, Fontemaggi G, Cong YS, Wen J, Hirte HW, Piaggio G, Sacchi A, Pontecorvi A, Bacchetti S, Farsetti A. Induction of hTERT expression and telomerase activity by estrogens in human ovary epithelium cells. *Mol Cell Biol*. 2000;20:3764–3771.
198. Komarov PG, Komarova EA, Kondratov RV, Christov-Tselkov K, Coon JS, Chernov MV, Gudkov AV. A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy. *Science*. 1999;285:1733–1737.
199. Bordone L, Guarente L. Calorie restriction, SIRT1 and metabolism: understanding longevity. *Nat Rev Mol Cell Biol*. 2005;6:298–305.
200. Longo VD, Kennedy BK. Sirtuins in aging and age-related disease. *Cell*. 2006;126:257–268.
201. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature*. 2003;425:191–196.
202. Libina N, Berman JR, Kenyon C. Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. *Cell*. 2003;115:489–502.