Epidemiological studies have shown that age is the chief risk factor for lifestyle-related diseases such as cardiovascular disease and diabetes, but the molecular mechanisms that underlie the increase in the risk of such diseases conferred by aging remain unclear. 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, the histology of human atherosclerotic lesions has been extensively studied, and it has been demonstrated that both vascular endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) exhibit the morphological features of cellular senescence, which suggests the occurrence of vascular cell senescence in vivo.

In fact, this hypothesis has been confirmed by in vivo cytochemical analysis of SAβ-gal activity. Fenton et al detected SAβ-gal-positive vascular cells in damaged rabbit carotid arteries. After repeated endothelial denudation, accumulation of SAβ-gal-positive cells was markedly enhanced. My group has previously demonstrated SAβ-gal-positive vascular cells in atherosclerotic plaques obtained from the coronary arteries of patients with ischemic heart disease. These SAβ-gal-positive cells were predominately localized on the luminal surface of the atherosclerotic plaques and were identified as ECs, but in the same patients such cells were not observed in the internal mammary arteries where atherosclerotic changes were minimal. In advanced plaques, however, SAβ-gal-positive VSMCs have been detected in the intima and not in the media, which may have been related to extensive cell replication in the lesions, as is observed in arteries subjected to double denudation. SAβ-gal-positive cells in human atheroma exhibit increased expression of p53 and p16, which is further evidence in favor of senescence. These cells also show various functional abnormalities, such as decreased expression of endothelial nitric oxide synthase (eNOS) and increased expression of pro-inflammatory molecules. Thus, cellular senescence may contribute to the pathogenesis of vascular aging in humans.

**Vascular Cell Senescence**

Vascular cells have a finite lifespan in vitro and eventually enter a state of irreversible growth arrest called cellular senescence. Flattening and enlargement of vascular cells are their morphological characteristics of senescence. Expression of negative regulators of the cell cycle (such as p53 and p16) increases with cell division and thereby promotes growth arrest. Primary cultured cells that undergo senescence in vitro also show increased expression of β-galactosidase (β-gal) activity at pH 6, which is distinguishable from the endogenous lysosomal β-gal activity that can be detected at pH 4. The activity at pH 6 is known as senescence-associated β-gal (SAβ-gal) activity, and because it shows a correlation with the aging of cells it is regarded as a biomarker of cellular senescence. In vitro growth of vascular cells obtained from human atherosclerotic plaques is impaired, and such cells develop senescence earlier than cells harvested from normal vessels. The histology of human atherosclerotic lesions has been extensively studied, and it has been demonstrated that both vascular endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) exhibit the morphological features of cellular senescence, which suggests the occurrence of vascular cell senescence in vivo.

**Telomere Shortening in Aged Arteries**

Telomeres are non-nucleosomal DNA-protein complexes located at the ends of chromosomes, serving as protective caps and acting 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. Telomerase is an enzyme that adds telomeres to the ends of chromosomes. In contrast to stem cells, which have high telomerase activity and maintain telomere length, most somatic cells, including vascular cells, show progressive telomere shortening because of low telomerase activity. Critical short telomeres resemble damaged DNA and thus trigger cellular senescence via a p53-dependent pathway. Recent studies have demonstrated that nuclear foci that contain markers of double-strand DNA...
breaks form in cells with critically short or dysfunctional telomeres, and these telomere dysfunction-induced nuclear foci are increased in the fibroblasts of aging primates.

It has been reported that telomere shortening occurs in human vessels and may be related to atherogenesis. Telomere length in ECs from the abdominal aorta and the iliac arteries shows a strong inverse correlation with age. Interestingly, telomere shortening occurs faster in the endothelial cells of the iliac arteries than in those of the internal mammary arteries. Thus, a high level of hemodynamic stress may enhance EC turnover in the iliac arteries compared with vessels subjected to less stress. Telomeres are shorter in coronary artery ECs from patients with coronary heart disease than in cells from healthy subjects. A recent study demonstrated that endothelial telomere length was shorter in patients with a longer history of risk factors for cardiovascular disease, suggesting that these factors override the effect of chronological aging on EC turnover by accelerating stress-induced damage. Identification of factors that accelerate endothelial telomere attrition will provide a novel target for the treatment of human atherosclerosis.

**Role of Telomeres in Vascular Senescence**

It has been demonstrated that disturbance of telomere integrity leads to endothelial dysfunction in vitro. Human ECs and VSMCs express telomerase activity, which is markedly increased by mitogenic stimuli, but this activity declines with aging because of decreased expression of the catalytic component of telomerase, leading to telomere shortening and cellular senescence. Introduction of telomerase prevents endothelial dysfunction associated with senescence, including decreased eNOS activity and increased monocyte adhesion to ECs. Immortalized human ECs have been established by introduction of telomerase, and these appear to retain EC characteristics, including various cell surface markers. When cultured in Matrigel, they form capillary-like structures as efficiently as ECs from an early passage.

Telomerase-deficient mice have a normal phenotype in the first generation, presumably because mice possess very long telomeres. However, their telomeres become shorter with successive generations, and the mice become infertile by the 6th generation because of impairment of the reproductive system. Some of the abnormalities in the later generations of these mice mimic age-associated changes. For example, these animals have a shortened lifespan and a reduced capacity to respond to stresses such as wounds and hematopoietic ablation. Neovascularization is also impaired in the later generations of telomerase-deficient mice, and their decreased ability to form new vessels may be attributed to impaired function and replication of vascular ECs 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 the reduced proliferation of macrophages. However, telomerase-deficient mice develop atherosclerotic plaques with a thin fibrous cap, suggesting that shortening of the telomeres in vascular cells may lead to plaque rupture in human atherosclerosis. Mice lacking telomerase activity develop hypertension in the 1st and 3rd generations as a result of an increased plasma endothelin-1 level caused by an overexpression of endothelin-converting enzyme.

**Stress-Induced Premature Senescence**

In response to various stress signals, cells develop a phenotype indistinguishable from that of senescent cells at the end of their replicative life span. For example, the constitutive activation of mitogenic stimuli by expression of oncogenic Ras induces a senescent phenotype in vascular cells.
Senescence triggered by mitogenic stimuli is independent of replicative age, and these signals act before the replicative limits of cells. Hence, it is apparently telomere-independent and thus termed ‘stress-induced premature 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. Ang II has been reported to induce the premature senescence of human VSMCs via the p53/p21-dependent pathway. Ang II was 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. Loss of p21 markedly ameliorated the induction of proinflammatory molecules by Ang II, thereby preventing the development of atherosclerosis.

Oxidative stress and DNA damage have been shown to induce premature senescence in vascular cells and have been suggested to contribute to atherogenesis. There is also evidence that exposure to chronic oxidative stress, including oxidized low-density lipoprotein, enhances telomere shortening and accelerates the onset of senescence in human ECs. Conversely, treatment with antioxidants preserves telomere length and extends the lifespan of ECs isolated from patients with severe coronary heart disease, unless the oxidative stress-induced damage becomes irreversible. One of the cellular targets of oxidative stress is DNA. Many different types of oxidative DNA lesions have been described, ranging from base modifications to single- and double-strand breaks. To cope with DNA damage, cells have evolved repair systems. Mice that lack components of these DNA repair systems exhibit the early onset of changes associated with aging, similar to their human counterparts, and fibroblasts from these mice show accelerated senescence. Constitutive activation of p53 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. Together with the data from telomerase-deficient mice, these results provide in vivo evidence of a link between cellular senescence and aging of the organism.
Cardiac Senescence

It has been reported that the number of myocytes declines with advancing age, presumably because of apoptosis or necrosis, whereas human cardiomyocyte replication has been reported to occur in the failing heart, as well as in the infarcted heart, suggesting that cardiac homeostasis could be regulated by the balance between myocyte loss and proliferation, and that aging impairs this equilibrium. In line with this notion, the number of myocytes with short telomeres is increased in the aging rat heart. Likewise, telomere length is significantly reduced in the cells of the human failing heart compared with normal samples. This reduction is enhanced with aging and associated with increased cardiac apoptosis, as well as activation of the DNA damage checkpoint kinase Chk2. Later generations of telomerase-deficient mice show significant telomere shortening in myocytes, which is coupled with attenuation of myocyte proliferation and increased apoptosis. These impairments are associated with ventricular dilatation and systolic dysfunction, suggesting that telomere shortening with age could contribute to cardiac failure in the elderly. Cardiac telomerase activity declines with age, whereas forced expression of telomerase prevent age-associated telomere shortening, thereby promoting cardiomyocyte proliferation and survival. Telomerase is detected mostly in cycling myocytes that express stem cell antigens, but also in proliferating myocytes without stem cell markers. The number of these proliferative cardiomyocytes is increased in human cardiac hypertrophy and ischemic heart failure, however; regeneration of myocytes appears to be insufficient, resulting in systolic dysfunction. Recently, a genetic fate-mapping study has demonstrated that cardiac progenitor cells (CPC) participate in the formation of new cardiomyocytes after injury, but do not contribute to refreshment of uninjured cardiomyocytes during normal aging in mice. Accumulating evidence has suggested that aging or pathological stimuli promotes both senescence and apoptosis of CPC, thereby decreasing the number of functionally competent CPC. For example, the number of p53- or p16-positive CPC with short telomeres is increased in the aged animal heart, as well as in the human chronic ischemic heart. Senescence of CPC is more prominent in old patients with a diseased heart. In the hearts of diabetic subjects, increased oxidative stress leads to telomere shortening, upregulation of p53 and p16, and apoptosis of CPC, thereby compromising cardiac structure and function. Together with senescent CPC, old non-replicating p53/p16-positive myocytes with short telomeres are increased in the aged heart, and these cells exhibits impaired contractile function. Thus, impaired function of senescent CPCs appears to affect the refreshment of myocytes, and therefore senescent myocytes with poor contractility accumulate, leading to the development of aging myopathy. Activation of CPC by growth factors, such as insulin-like growth factor-1 and hepatocyte growth factor, has been shown to promote regeneration of cardiomyocytes, restoring the cardiac dysfunction associated with aging, suggesting that treatment with these growth factors may become an effective therapeutic strategy for myocardial aging.
Cardiac hypertrophy is an adaptive response to increased workload in order to maintain cardiac function. However, prolonged cardiac hypertrophy causes heart failure, and the mechanisms are largely unknown. It was recently demonstrated that cardiac angiogenesis is critically involved in the adaptive mechanism of cardiac hypertrophy and that p53 accumulation is crucial for the transition from cardiac hypertrophy to heart failure. Pressure overload initially promotes vascular growth in the heart as a result of hypoxia-inducible factor-1 (HIF-1)-dependent induction of angiogenic factors, and inhibition of angiogenesis prevents the development of cardiac hypertrophy and induced systolic dysfunction. Sustained pressure overload induces accumulation of p53, which inhibits HIF-1 activity and thereby impairs cardiac angiogenesis and systolic function. Conversely, promoting cardiac angiogenesis by introducing angiogenic factors or by inhibiting p53 accumulation further develops hypertrophy and restores cardiac dysfunction under chronic pressure overload. These results suggest that the anti-angiogenic property of p53 plays a critical role in the transition from cardiac hypertrophy to heart failure (Figure 2).

Accumulation of p53 in the heart has been reported in aging and several diseases, such as diabetes, which may be the reason older people and diabetic patients are susceptible to heart failure when chronic pressure overload develops. Thus, inhibition of p53 or promotion of vascular growth in the heart may be a novel therapeutic strategy to prevent the transition from cardiac hypertrophy to heart failure in aged people and diabetic patients.

Adipose Senescence and Diabetes

Aging is known to increase the prevalence of metabolic disorders such as diabetes. Therefore, it has been hypothesized that cellular aging might influence insulin resistance (IR) and accelerate the development of diabetes. Using various genetic models, including telomerase-deficient mice, it has been recently shown that p53 in adipose tissue is critically involved in IR, which underlies age-related cardiovascular and metabolic disorders. Telomerase-deficient mice with short telomeres developed IR when fed a high-calorie diet. The adipose tissue of these mice showed senescence-like changes, such as increases in the activity of SA-β-gal, level of expression of p53, and production of proinflammatory cytokines. Resection of senescent adipose tissue improved IR in the telomerase-deficient mice, and implantation of senescent adipose tissue into wild-type mice led to impairment of insulin sensitivity and glucose tolerance in the recipients. Upregulation of p53 induced the expression of proinflammatory cytokines and accumulation of macrophages in adipose tissue. It was also found that excessive calorie intake led to the accumulation of oxidative stress in the adipose tissue of type 2 diabetic mice and promoted senescence-like changes, thereby increasing production of pro-inflammatory cytokines. Inhibition of p53 activity significantly ameliorated these senescence-like changes of adipose tissue, decreased the expression of pro-inflammatory cytokines, and improved IR in type 2 diabetic mice. Conversely, upregulation of p53 in adipose tissue caused an inflammatory response that led to IR. Adipose tissue from diabetic patients also shows senescence-like features. These findings indicate that cellular aging signals (particularly p53 in adipose tissue) upregulate the expression of proinflammatory molecules, thereby promoting the infiltration of macrophages into adipose tissue, which leads to a further increase in the production of proinflammatory cytokines by the adipose tissue, which induces IR and glucose intolerance (Figure 3).

Our results demonstrate a previously unappreciated role of adipose-related p53 in the regulation of IR and suggest that cellular aging signals in adipose tissue could be a novel target for the treatment of diabetes.

Recent studies have shown that longevity signals in adipose tissue plays a crucial role in regulating the lifespan of various species, ranging from worms to mice, and suggested the existence of cellular non-autonomous regulation of aging by adipose tissue. Consistent with those reports, subcutaneous implantation of senescent adipose tissue from telomerase-deficient mice accelerated the senescence of epididymal fat in wild-type recipients. The senescence of adipose tissue may increase the local production of proinflammatory molecules, but may also promote systemic inflammation via cellular non-autonomous mechanisms. Low levels of circulating insulin are generally associated with longevity, and activation of longevity signals in adipose tissue has been reported to reduce the circulating insulin level and extend the lifespan. It has been found that inhibition of p53 activity in adipose tissue improves IR and thus decreases the plasma insulin level. Thus, p53 activation in adipose tissue may be a pro-aging signal that negatively regulates longevity, so inhibition of cellular aging may become a novel therapeutic strategy for aging and its associated diseases.

Conclusions

Cell division is essential for the survival of multicellular organisms that contain renewable tissues, but places the organism at risk of developing cancer. Thus, complex organisms have evolved at least 2 cellular mechanisms to prevent oncogenic transformation: apoptosis and cellular senescence. In this regard, aging and age-associated diseases can be viewed as byproducts of the tumor suppressor mechanism known as cellular senescence. Consistent with this idea, the number of senescent fibroblasts increases exponentially in the skin of aging primates. Conversely, extension of the lifespan by calorie restriction decreases biomarkers of cellular senescence in vivo. We therefore need to identify the molecular mechanisms by which aging accelerates cellular aging in order to prevent the development of lifestyle-related diseases. Identification of such mechanisms could lead to a new treatment strategy for various age-associated diseases, such as neurodegenerative disease, as well as lifestyle-related diseases.

References


