Aging and Atherosclerosis
Mechanisms, Functional Consequences, and Potential Therapeutics for Cellular Senescence

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Abstract: Atherosclerosis is classed as a disease of aging, such that increasing age is an independent risk factor for the development of atherosclerosis. Atherosclerosis is also associated with premature biological aging, as atherosclerotic plaques show evidence of cellular senescence characterized by reduced cell proliferation, irreversible growth arrest and apoptosis, elevated DNA damage, epigenetic modifications, and telomere shortening and dysfunction. Not only is cellular senescence associated with atherosclerosis, there is growing evidence that cellular senescence promotes atherosclerosis. This review examines the pathology of normal vascular aging, the evidence for cellular senescence in atherosclerosis, the mechanisms underlying cellular senescence including reactive oxygen species, replication exhaustion and DNA damage, the functional consequences of vascular cell senescence, and the possibility that preventing accelerated cellular senescence is a therapeutic target in atherosclerosis. (Circ Res. 2012;111:245-259.)

Key Words: aging ■ atherosclerosis ■ senescence ■ DNA damage ■ vascular smooth muscle

Atherosclerosis and the subsequent cardiovascular complications, such as myocardial infarction, stroke, and ischemic heart failure, is a major cause of death in the Western world. The risk factors of atherosclerosis are well known, including hypertension, diabetes, serum total and low-density lipoprotein (LDL) cholesterol, and smoking. Increasing evidence indicates that aging is also an important risk factor for atherosclerosis and persists as an independent contributor when all other known factors are controlled. Premature or accelerated vascular aging can be promoted by cardiovascular risk factors, and cellular senescence is also observed in patients with atherosclerosis. Atherosclerosis is therefore a disease of both organismal aging and cellular senescence par excellence.

Pathology of the Advanced Atherosclerotic Plaque
The mature atherosclerotic plaque comprises an accumulation of vascular smooth muscle cells (VSMCs) and their secreted products (collagen and elastin), inflammatory cells (macrophages, T lymphocytes, dendritic cells, and mast cells), and both intracellular and extracellular lipid and debris (Figure 1). The latter is often concentrated in a necrotic core surrounded by a fibrous cap composed predominately of VSMCs. The stability of the atherosclerotic plaque depends on the thickness of the fibrous cap and the degree of cap inflammation. Plaque rupture is increased by cap thinning, promoted by death of VSMCs and breakdown of collagen and extracellular matrix (ECM), which may subsequently lead to myocardial infarction or stroke. However, plaque rupture is frequently subclinical, as VSMCs repair the rupture and reorganize associated thrombus. Indeed, complicated plaques frequently show evidence of multiple ruptures and repair, ultimately resulting in luminal narrowing. Successful plaque repair requires VSMCs to proliferate and synthesize matrix, both properties that are altered by cellular senescence. Indeed, cellular senescence can alter many of the proatherogenic events seen in Figure 1.

Pathology of Normal and Premature Vascular Aging
Vessel aging, even in the absence of atherosclerosis, leads to intimal and medial thickening (vascular remodeling) as well as gradual loss of arterial elasticity, resulting in vascular stiffness. Aged vessels show a number of characteristic pathological processes, many of which are also seen in atherosclerosis. For example, aged vessels show reduced medial VSMC number, increased collagen deposition, and fracture of the elastin lamellae, which may lead to vessel dilation and increased lumen size. Increased collagen and decreased elastin content, promoted at least in part by age-associated increases in glycated proteins, matrix metalloproteinase enzyme activity, and trophic stimuli such as angiogenesis II signaling, impair vessel elasticity and hence promote vascular stiffness and subsequently hypertension.
Hypertension can further stimulate collagen production with increased vessel stiffness and endothelial cell (EC) dysfunction. In addition to alterations in matrix and cell composition, aged vessels show elevated expression of a number of proinflammatory molecules and increased uptake of plasma lipoproteins. In part, these effects may be due to increased expression of leukocyte adhesion molecules on ECs in aged vessels, which trigger the familiar processes of monocyte migration followed by increased uptake of atherogenic lipoproteins with subsequent inflammation, key events that ultimately promote atherosclerosis (Figure 1). Aged ECs and VSMCs also show increased secretion of proinflammatory cytokines (see below), resulting in persistent vascular inflammation. Thus, the effects of atherosclerosis are superimposed on normal aging of the underlying vessel.

A number of genetic diseases associated with premature aging also show the typical pathology of vascular aging and are prone to cardiovascular complications including atherosclerosis. For example, Hutchinson Gilford progeria syndrome (HGPS) is a rare, fatal, and progressive premature aging condition due to defects in lamin A that forms nuclear lamina. HGPS vessels from young patients reveal accumulated collagen, fractured elastin lamellae, and a thickened intima, with some vessels showing advanced atherosclerotic lesions containing chronic inflammation, calcification, and VSMC loss. Mouse models of HGPS recapitulate the vascular pathology in patients and also show increased vascular stiffness and hypertension, supporting the concept of premature vascular aging. Another example is Werner syndrome (WS), a loss-of-function mutation in the WS syndrome (WS), a loss-of-function mutation in the WS ATP-dependent helicase (WRN), which shows a similar pathology and accelerated atherosclerosis. WS knock-out mice also show insulin resistance and increased blood glucose, increased circulating cholesterol, and enhanced lipid deposition, indicating that intrinsic vessel aging is superimposed on metabolic effects to promote vascular disease. HGPS and WS serve as important examples to understand any causal relationship between vascular disease and aging.

Because atherosclerosis compounds the pathological changes associated with normal vascular aging and vascular aging promotes atherosclerosis, this brings up the “chicken and egg” argument—does biological aging promote atherosclerosis, or does atherosclerosis promote vessel aging and cellular senescence? We suggest that these scenarios are not mutually exclusive but both occur simultaneously. While both structural changes associated with aging and cellular senescence are associated with atherosclerosis, this review focuses predominately on the latter, particularly on VSMC senescence, although other cell types undergo the same processes after the same stimuli. We will outline evidence of cellular senescence, proposed mechanisms, as well as current therapeutic options targeting vascular aging or cellular senescence in atherosclerosis.

**Evidence of Cellular Senescence in Atherosclerosis**

There are marked changes in multiple cell types in older versus younger individuals, including VSMCs, ECs, and inflammatory cells. Prematurely and naturally aged cells share several characteristics, including changes in cell
proliferative potential, markers of cell senescence, increased propensity to undergo cell death, elevated DNA damage, and extensive telomere shortening and dysfunction. All of these features can be detected in cells from atherosclerotic plaques, which show additional features of cell senescence.

**Cell Proliferation**

VSMCs in the normal vessel wall have a very low turnover, with barely measurable proliferation indices. Increased cell proliferation is observed during early atherogenesis and on vascular injury,18 and aged VSMCs from rodents also show increased proliferation14,15,19 compared with cells from younger animals. In contrast, human VSMCs derived from both aged vessels and advanced atherosclerotic plaques undergo reduced proliferation and prolonged population doubling times.20,21 This observation corresponds to in vitro findings in which plaque VSMCs in culture show decreased percentages in S-phase and increased percentages in G1, consistent with a G1 growth arrest.21 While some of the arrest is associated with reduction in responses to mitogens, such as insulin-like growth factor 1 (IGF-1),22 the arrest is mediated by major changes in the expression of various cell cycle regulators, especially those involved in G1-S transition. Thus, increased expression of the cyclin-dependent kinase inhibitors p16INK4a and p21,23 decreased cyclin D and cyclin E,24 and hypophosphorylation of the retinoblastoma protein (pRB)23–25 are observed in both normal human VSMCs undergoing replicative senescence and human plaque VSMCs. Plaque VSMCs also show reduced expression of the transcription factors E2F1–3 and increased sequestration of E2F1 to pRB.23 Importantly, these cell cycle regulators become potential markers of vascular cell senescence.
Although most of these studies have examined cells that are presumed to be derived from the vessel wall itself, a number of published works suggest that at least a proportion of cells expressing VSMC or EC markers may be derived from bone marrow–derived progenitor cells (BMCs) and endothelial progenitor cells (EPCs) that migrate and integrate into the vessel wall and may themselves be affected by aging. This is a very controversial area, but dysfunctional EPCs and impaired BMC migration and adhesion are seen in both aged and atherosclerotic mouse models. These cells from aged animals are accompanied by reduced expression of cell surface markers and cytokines for chemotaxis, such as C-X-C chemokine receptor type 4, decreased hypoxia-inducible factor 1A, and as well as increased oxidative stress and inflammation.

Cell Senescence

Cell senescence is defined as the irreversible loss of the ability of cells to divide. There are 2 general types of cell senescence, replicative senescence and stress-induced premature senescence (SIPS). Replicative senescence occurs with exhaustion of proliferative lifespan over time, a characteristic of aging, and is associated with critically shortened telomeres at chromosomal ends, which then induce a DNA damage response (DDR) (see below). In contrast, SIPS is triggered by external stimuli, including oxidizing agents and radiation, which activate the intracellular senescence cascade prematurely. While SIPS shares many morphological and molecular characteristics to replicative senescence, SIPS is not usually characterized by telomere shortening.

As well as altered expression of cell cycle regulators, senescent cells are characterized by “specific” markers, including senescence-associated β galactosidase (SAβG), a lysosomal enzyme seen in senescence of multiple human cell types. Increased numbers of SAβG-positive VSMCs, ECs, and monocyte/macrophages are observed in aged vessels and atherosclerotic lesions when compared with their respective young and normal counterparts, reinforcing the idea that atherosclerosis is associated with premature cellular senescence. However, a word of caution is required when interpreting SAβG staining. In particular, cells with a high lysosomal content, such as macrophage foam cells, show SAβG reactivity that may not reflect senescence.

Apoptosis

Although cell death through multiple processes (necrosis, autophagy, apoptosis) occurs in atherosclerosis, the best described in association with senescence is apoptosis. Apoptosis, identified by morphological and molecular changes, is increased in aged vascular cells and is also increased in VSMCs and other cells in atherosclerotic plaques. While apoptosis occurs in ECs, VSMCs, and macrophages, most cell death in plaques is within the macrophage-rich necrotic core of the plaque. However, many apoptotic cells lose their lineage markers, which makes their precise identity difficult to be determined.

Telomere Shortening

Telomeres consist of tandem repeats of the sequence TTAGGG at the end of chromosomes. At birth, telomere lengths in most tissues from the same subject are similar. Due to the mechanism of DNA replication, telomeres shorten with each cell division in cells that lack high activity of specific enzymes, such as telomerase, which maintain telomere length. Accelerated telomere shortening is also associated with dysregulation and dysfunction of proteins involved in telomere maintenance. Telomere length thus can be used to track a tissue’s replication history and has been used as a surrogate for “biological” age.

Telomere length and integrity is regulated through the interplay between telomerase, an enzyme composed of a RNA template (TERC) and catalytic subunit (TERT), and a complex of proteins known as Shelterin, consisting of TRF1, TRF2, Rap1, TIN2, POT1, and POT1, which pack the telomere into a so-called “T-loop.” The compact T-loop structure blocks the access of endonucleases required for telomere recombination and of DDR proteins that read telomeres as DNA double-stranded breaks. The T-loop can also regulate the access of telomerase to telomeres without affecting its expression and activity. Indeed, increasing evidence suggests that telomere integrity rather than telomere length is responsible for controlling cell longevity. In particular, TRF2, a member of the Shelterin complex, may be a master regulator stabilizing the complex at telomeres and maintaining the compact T-loop. Cells overexpressing TRF2 continue to proliferate regardless of telomere length in vitro. In contrast, cells with mutated TRF2 show aberrant chromosome structures, including chromosome end-fusions and shorter telomeres, which are sufficient to induce premature senescence and apoptosis.

Shortened telomeres are evident in atherosclerosis, observed in plaque VSMCs and ECs relative to the normal vessel wall, and in circulating EPCs. Also, telomeres in leukocytes are shorter in patients with atherosclerosis compared with control subjects and are inversely correlated to cardiovascular disease risks in patients with subclinical diseases. Short telomeres and low levels of telomerase expression and activity are functionally important in VSMC senescence, as ectopic telomerase expression can dramatically increase lifespan of both plaque and normal VSMCs. However, some of these effects may be independent of telomeres, as telomeres continue to shorten in these cells and cells replicate with critically short telomeres. In addition, whereas telomere length mostly reflects previous replication, arterial segments resistant to atherosclerosis, such as internal mammary artery or ascending aorta, have longer telomeres than the aortic regions prone to the disease. This difference is age-independent, suggesting the existence of intrinsic genetic or developmental variations in telomere regulation may underlie location-specific predisposition in atherogenesis.

The ease that telomere length can be measured has resulted in an explosion in studies associating telomere length with multiple diseases or preclinical disease. However, there are important caveats to the slavish adoption of telomere length as a biomarker for aging in disease, or in animal models. Importantly, telomere length is relatively stable over time between 20 to 70 years of age in humans when vascular aging is apparent; telomere length rarely reaches levels classified as
“critical” to affect telomere function in cardiovascular studies, making it unclear whether the cells with shorter telomere length have any discernable loss of function; and telomeres in animal models (especially rodents) have a very different structure to those in humans. Finally, unlike other biomarkers of disease activity, successful treatment of cardiovascular disease is highly unlikely to increase telomere length, although it might slow loss of telomeres.

DNA Damage
Aside from shortened telomeres, VSMCs, ECs, macrophages, and circulating cells from the elderly and patients with atherosclerosis contain increased DNA damage in both nuclei and mitochondria compared with younger subjects and those without vascular disease. The accumulation of DNA damage in cells may reflect both ongoing damage-inducing stimuli and deficits in the repair machinery. Similar to telomere shortening, DNA damage eventually leads to cell senescence and apoptosis, although minor damage is associated with transient growth arrest as repair is undertaken. DNA damage includes both single- and double-stranded breaks, deleted sections of DNA, nucleotide modifications, and extrusions of DNA from the nucleus (micronuclei), accompanied by altered expression of various DDR proteins. In brief, the DDR is initiated by the identification of “faulty” DNA, either a mismatch base or single- or double-stranded breaks. Identification and signaling is achieved by recruiting various sensor proteins, including phosphorylated forms of ataxia telangiectasia mutated (P-ATM) and histone 2A protein X (γH2AX) (Figure 2). Expression of both P-ATM and γH2AX increase with atherosclerotic plaque grade in vivo, and both markers are elevated in plaque VSMCs in vitro compared with cells from normal vessels. P-ATM and γH2AX are therefore used as common markers to identify DNA damage and the DDR in vivo. Recruitment of these proteins triggers an intracellular kinase cascade leading to the activation of a range of effectors to participate in DNA repair, including p53 and Chk2. The normal response is transient cell cycle arrest and repair of the damaged DNA. However, when DNA damage is too extensive to be repaired or when the repairing cascades are impaired, cell senescence and apoptosis occur.

DNA damage in mitochondria is also seen in atherosclerosis, characterized by a 4977 bp deletion. This deletion is associated with mitochondrial dysfunction, although it is not clear whether the levels of the deletion observed in atherosclerosis are sufficient alone to cause mitochondrial dysfunction. Whereas gradual loss of mitochondrial function occurs with age, it can be accelerated by oxidative stress, for example due to increased oxidized LDL (oxLDL) during atherogenesis. Epigenetic modifications are important in regulating gene silencing and activation. Age-related changes in methylation and acetylation patterns in the genome have been reviewed intensively and are linked to various diseases of premature aging, including atherosclerosis. Epigenetic changes are therefore used increasingly as markers of premature cellular senescence in the vasculature.

Although DNA methylation patterns are heterogeneous between sex, race, and age that make them challenging to use as biomarkers for atherogenesis, recent studies have identified changes in the expression of methyltransferases and hence in methylation in plaques and leukocytes from both patients and atherogenic mice. Altered methyltransferase expression correlates with hypomethylation of normally hypermethylated genomic regions and vice versa, which can occur within genes participating in proliferation, apoptosis and lipid metabolism. For example, hypomethylation corresponds with phenotypic modulation and proliferation in VSMCs; changes in methylation occur before overt atherosclerosis in mice models; and atherogenic lipoproteins can enhance DNA methylation and histone acetylation. These findings suggest a potential feedback loop between epigenetic modification and atherosclerosis.

Histone acetylation and deacetylation are also important contributors to atherosclerosis and aging, through which inflammation, VSMC proliferation, and ECM composition can be modulated. A well-studied example is the mitochondrial gene p66(Shc), also known as a longevity gene. Increased hypermethylation and histone acetylation result in the age-related enhancement of p66(Shc) production and hence the subsequent availability for activation. In contrast, deletion of p66(Shc) is atheroprotective by reducing oxidative stress, endothelial dysfunction, and vascular cell apoptosis.

Mechanisms of Cellular Senescence in Atherosclerosis
The mechanisms underlying cellular senescence in atherosclerosis are likely to be multiple and cumulative in any one cell or artery. For example, telomere shortening leading to replicative senescence will interact with nuclear and mitochondrial DNA damage due to free radicals, which themselves will be increased where there are defects in DNA repair. DNA damage may also interact with epigenetic modifications to regulate genes that themselves regulate cell proliferation, cell senescence, and apoptosis. For simplicity, we will outline each mechanism in turn and also provide evidence that each may be causal in atherosclerosis.

Telomere Maintenance
As described above, telomere shortening in vascular cells occurs with replication and is seen in plaques. Telomere shortening of circulating leukocytes also occurs with aging and in atherosclerosis, probably due to gradual bone marrow failure with telomere shortening of progenitor cells. Telomere length may therefore have functional consequences in aging and atherosclerosis. In contrast, whereas ectopic telomerase can maintain cell proliferation and reverse the tissue degeneration in TERT-deficient mice, reduction of telomerase activity in TERC−/− mice was found to be atheroprotective, linked to decreased proliferative potential in macrophages and other inflammatory cells. These opposing findings suggest that telomerase activity may affect athero-
Figure 2. Model of premature cellular senescence in atherosclerosis. Cardiovascular risk factors including genetic predisposition accelerate normal biological aging of vessels, resulting in telomere attrition, accumulated DNA damage, increased oxidative stress, and epigenetic modifications. Inherited defects in DNA repair enzymes or lamin also accelerate this process. While epigenetic modifications lead to transcriptional alterations that can change cell function, activation of the DNA damage response (DDR) is a common result. DDR sensors, such as ATM and H2AX, are phosphorylated and bind to damaged DNA regions, followed by recruitment of various DDR proteins including MRE11 and NBS1. These signaling pathways activate various downstream effectors, such as p53 and Chk2, leading to temporary growth arrest for DNA damage repair. Successful repair allows cells to continue proliferating and repair vessel damage. In contrast, unsuccessful DNA repair results in accumulation of both nuclear and mitochondrial DNA damage, and cells undergo telomere-dependent and telomere-independent senescence and apoptosis and secrete proinflammatory cytokines as part of the SASP. These processes lead to the loss of normal cellular function and increased inflammation seen in aging and in atherosclerotic plaques. Cellular dysfunction contributes to the alterations in ECM proteins, resulting in vascular stiffness and loss of elasticity, and the proinflammatory state promotes atherosclerosis. Atherosclerosis may also contribute directly to accelerated vascular aging, as vascular repair can promote replicative senescence, and the proinflammatory state and ROS promote stress-induced premature senescence (SIPS) (illustration: Cosmocyte/Ben Smith).
sclerotic development in a stage-dependent manner or that manipulation of telomere length by associated proteins may affect different cells differently. Indeed, dysfunction of the Shelterin protein complex has different effects on macrophages compared with lymphocytes. In addition, telomerase overexpression cannot always prevent and/or delay cell senescence, as observed in both VSMCs and ECs. This suggests that telomere-independent mechanisms, such as increase in genomic instability and mitochondria dysfunction, may contribute to the effects of DNA damage in atherosclerosis.

DNA Damage Recognition and Repair

DNA damage occurs naturally by both intrinsic (for example, spontaneous mutation and replication errors) and extrinsic (for instance, UV radiation, chemical exposure, and free radicals) stimuli. Although DNA damage and DDR activation are seen in atherosclerosis, increasing evidence from studies of DDR proteins and repair enzymes indicates that DNA damage directly promotes both atherosclerosis and medial degeneration. For example, ATM heterozygosity accelerates atherosclerosis in mouse models, accompanied by increased nuclear and mitochondrial DNA damage and metabolic changes similar to the metabolic syndrome. These phenotypes can be partially rescued by bone marrow transplant with wild-type ATM. Similarly, age-associated accumulation of prelamin A disrupts normal compartmentalization of nuclear signaling pathways and leads to insufficient DNA repair, which in part contributes to DNA damage accumulation and accelerates atherosclerosis. As described above, defects in lamin A are also found in HGPS patients, whose cells contain increased DNA damage and who show signs of premature aging including atherosclerosis. Moreover, cells from patients with WS and the representative knock-out animal models show genetic instability. Thus, defective DNA repair directly promotes atherosclerosis.

Reactive Oxygen Species and Oxidative Stress

In vitro studies have shown that in comparison to VSMCs from younger subjects, aged VSMCs contained greater oxidative stress-induced damage, probably due to the combination of higher reactive oxygen species (ROS) generation and impaired antioxidant defense. The major source of ROS within the cell is as a by-product of oxidative phosphorylation. Mitochondrial DNA damage and dysfunction can increase ROS generation, creating a positive feedback loop. ROS are also increased by elevated levels of oxidized lipoproteins in atherosclerosis, with the most common form being reactive hydroxyl free radicals (OH). Moreover, ROS production can be regulated through mechanical stress, as ECs under laminar shear stress upregulate antioxidant enzymes such as peroxiredoxins. Turbulent flow at sites of plaque formation would thus reduce antioxidant capacity.

ROS promote DNA damage by either adding double bonds to or removing the hydrogen atom from DNA bases. Oxidative DNA damage occurs in both mitochondrial and nDNA and in both telomeric and nontelomeric regions. Indeed, the level of 8-oxo-deoxyguanosine (8-oxodG), an oxidized form of guanine marking oxidative DNA damage, is elevated in VSMCs and macrophages in plaques, and, as discussed earlier, increased DNA damage and the subsequent DDR promote cell senescence and apoptosis. In addition, at higher concentrations, ROS can produce SIPS in vascular cells.

Apart from directly promoting DNA damage, ROS can modulate atherosclerosis progression by other mechanisms that also involve premature cellular senescence. For example, ROS can inactivate the PI-3K/Akt pathway, which can downregulate and inactivate telomerase and directly promote apoptosis. ROS-induced p53 activation, possibly indirectly via increasing the DDR, reduces the expression of the IGF-1 receptor in VSMCs and the subsequent Akt survival signals. ROS can also stimulate transforming growth factor-β (TGFβ) signaling by inducing the release of active TGFβ from its inactive secreted form, the latent transforming growth factor-β (LTGFβ). Furthermore, ROS can increase the expression of cell surface receptors required for uptake of oxLDL, a feature also seen in aging.

Epigenetic Modifications

Increasing evidence suggests that not only do epigenetic modulations involving changes in methylation, acetylation, and noncoding RNA occur in atherosclerosis, these changes regulate important processes in premature cellular senescence and atherosclerosis. For example, the monocarboxylate transporter (MCT)3, which participates in lactate transport in VSMCs, is hypermethylated in atherosclerotic lesions. As a consequence, MCT3 expression is reduced and is associated with VSMC proliferation. Similarly, estrogen receptors (ER) are expressed in both ECs and VSMCs. Hypermethylation of both receptor subunits is detected with normal aging and in patients with atherosclerosis. Hypermethylation with VSMC proliferation. Similarly, estrogen receptors (ER) are expressed in both ECs and VSMCs. Hypermethylation of both receptor subunits is detected with normal aging and in patients with atherosclerosis. Furthermore, ROS can increase the expression of cell surface receptors required for uptake of oxLDL, whereas hypermethylation of ERβ appears in advanced lesions and is involved in vascular senescence.

A number of miRNAs are upregulated in atherosclerosis and/or regulate processes associated with aging, such as cell proliferation, apoptosis, or senescence (summarized in Figure 3). For example, miR-21 is increased after vessel injury, promotes VSMC proliferation and regulates VSMC survival through modulation of phosphatase and tensin homolog and B-cell lymphoma-2 (Bcl-2) expression. Similarly, levels of miR-221/222 are elevated both in patients with coronary artery disease and after vessel injury. miR-221/222 is upregulated by PDGF and also promotes VSMC proliferation, in part by downregulation of p27kip. In contrast, the expression of miR-143/145 which are controlled by TGFβ signaling are repressed during neointimal formation on injury and in atherosclerotic development. Increased TGFβ in advanced stages of atherosclerosis enhances miR-143/145 expression, which subsequently inhibits Kruppel-like factor-4/5 (KLF4/5) and hence prevents VSMC proliferation. Increased cell cycle arrest and senescence in VSMCs can promote miR-133, which further represses VSMC proliferative phenotype and forms a feedback loop. In addition, overexpressing miR-143/145 at early point of vascular injury can limit neointimal formation, further
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Arterial aging has a significant genetic predisposition and is mediated at least in part through accelerated cellular senescence. For example, senescent ECs show reduced expression of miR-21, miR-214, and miR-92, accompanied by increased miR-221/222. Moreover, miRNAs can also modulate atherosclerosis by targeting genes involved in lipid metabolism. For instance, miR-33 suppresses the expression of the cholesterol transporter ABCA1, leading to reduced high-density lipoprotein (HDL) biogenesis and fatty acid oxidation. Antagonizing miR-33 activity is atheroprotective, accompanied by increased HDL and smaller plaques. Although these examples of changes in epigenetic markers and miRNA abundance are interesting, what is lacking is direct evidence that either is causally responsible for the changes in cell proliferation or senescence in normal or premature aging. This is clearly a future area for study.

**Systemic Causes**

The above discussion has focused on local stimuli for cellular senescence and vascular aging, outlining the cellular mechanisms involved. However, there are many systemic factors that predispose to vascular aging, including genetic predisposition, some of which are also risk factors for atherosclerosis. It is now becoming clear that their effects are mediated at least in part through accelerated cellular senescence and through the mechanisms outlined.

Arterial aging has a significant genetic predisposition and varies between races. For example, African-Caribbean individuals have accelerated arterial aging, increased aortic stiffness and greater carotid intima thickness when compared with European and Asian populations. This indicates the likely presence of genetic loci controlling multiple aspects of vessel aging. Similarly, age-related artery stiffness is more pronounced in women and is independent of the menopause, reflecting intrinsic differences in arterial properties between sexes. Although men have more prominent accelerated aging over time compared with women, there are complex effects of hormone deficiency and replacement therapy on arterial stiffness and incidence of nonfatal cardiovascular diseases that may be reversed on withdrawal. Thus, both genetic predisposition and sex affect vessel aging.

Acquired risk factors are also major contributors to premature vascular aging and cellular senescence. For example, smoking increases arterial stiffness and pulse pressure and promotes SIPS in ECs and fibroblasts. Similarly, whereas low to moderate and/or acute alcohol intake are associated with reduced arterial stiffness, chronic higher alcohol intake (>21 U/wk) results in opposing effects. Finally, age and diet-associated elevation of serum glucose is associated with both premature vascular stiffness and vascular cell senescence, which are particularly pronounced in younger patients and can be reduced by physiological levels of insulin. Glucose participates in a spontaneous, nonenzymatic reaction known as glycation, which adds sugars to proteins and lipids to form reactive advanced glycation end products (AGEs). Both glycation and AGEs increase with age and in patients with atherosclerosis and metabolic disorders and correlate with serum glucose levels. Multiple animal studies demonstrate the underlying mechanisms through which vascular aging is promoted by glycation, including promoting vascular stiffness through collagen stabilization and attenuating nitric oxide (NO) production in ECs, increasing vascular permeability and cell adhesion, enhancing VSMC migration, augmenting circulating lipoproteins for oxidation and their subsequent uptake by macrophages, and increasing inflammation and oxidative stress. Reducing dietary AGES can be atheroprotective and glycation can be modulated by other risks factors, for example by smoking.

**Functional Consequences of Cell Aging**

Aging-associated changes are observed in multiple cell types and are conserved across species, from rodents to primates. As well as loss of normal cellular function, these changes result in specific consequences in each cell type, which may be directly proatherogenic.

**Endothelial Cells**

Aged ECs become flatter, enlarged, and have an increasingly polypoid nucleus, all features associated with cellular senescence. These changes are accompanied by modulation in cytoskeleton integrity, proliferation, angiogenesis, and cell function. The above discussion has focused on local stimuli for cellular senescence and vascular aging, outlining the cellular mechanisms involved. However, there are many systemic factors that predispose to vascular aging, including genetic predisposition, some of which are also risk factors for atherosclerosis. It is now becoming clear that their effects are mediated at least in part through accelerated cellular senescence and through the mechanisms outlined. Arterial aging has a significant genetic predisposition and varies between races. For example, African-Caribbean individuals have accelerated arterial aging, increased aortic stiffness and greater carotid intima thickness when compared with European and Asian populations. This indicates the likely presence of genetic loci controlling multiple aspects of vessel aging. Similarly, age-related artery stiffness is more pronounced in women and is independent of the menopause, reflecting intrinsic differences in arterial properties between sexes. Although men have more prominent accelerated aging over time compared with women, there are complex effects of hormone deficiency and replacement therapy on arterial stiffness and incidence of nonfatal cardiovascular diseases that may be reversed on withdrawal. Thus, both genetic predisposition and sex affect vessel aging.
migration. Senescent ECs show attenuated endothelial NO production and increased endothelin-1 release. Late-passage ECs also show reduced expression of the adhesion molecules VCAM-1 and intracellular adhesion molecule-1 (ICAM-1), increased activation of nuclear factor (NF)-κB, and increased susceptibility to apoptosis. Moreover, there are marked age-associated changes in ICAM-1 function and activity. Thus, EC senescence is associated with loss of EC function and a shift toward a proinflammatory and proapoptotic state, predicted to enhance monocyte migration into the vessel wall.

**Vascular Smooth Muscle Cells**

VSMCs in human plaques or derived from plaques show reduced proliferation, early senescence, and increased susceptibility to apoptosis. These properties would reduce the ability to repair plaques that undergo rupture. Aged rodent aortas also show increased levels of interleukin (IL)-6 and aged aortic VSMCs have a higher basal secretion of IL-6 than young VSMCs. Indeed, secretion of a common set of secreted proteins as cells age is a widespread phenomenon known as the “senescence-associated secretory phenotype,” or SASP. Moreover, aged VSMCs exhibit upregulation of chemokines (chemokine CC-motif ligand 2), adhesion molecules (eg, ICAM-1), and innate immune receptors (eg, Toll-like receptor 4). These properties generate a proinflammatory environment, further promoting migration of inflammatory cells. VSMC senescence thus promotes atherosclerosis progression and inhibits plaque repair.

**Inflammatory Cells**

Monocytes from patients with atherosclerosis demonstrate an increased burst of free radicals on activation and increased secretion of a number of cytokines, including monocyte chemotactic protein (MCP)-1, IL-6, IL-1β, and tumor necrosis factor-α. Importantly, many of these differences are also observed in aged versus young monocytes and can be recapitulated by agents that disrupt telomeres. Thus, there is direct evidence that aging promotes proinflammatory changes in monocyte/macrophages that are relevant to atherosclerosis. Coupled with altered adhesion molecules on aged ECs, aging would be predicted to promote both migration and activation of macrophages within plaques.

**Targeting Aging in Atherosclerosis**

The association between aging and atherosclerosis and the increasing evidence demonstrating that accelerated cellular senescence occurs in vascular disease have resulted in the search for treatments that can promote longevity and delay senescence. Indeed, pharmaceutical companies are now developing drugs that target specific mechanisms of premature aging, including telomerase activity, DNA methylation, DNA damage repair, and miRNA. Specific agents may also target the DNA damage machinery and DDR directly. For example, chloroquine has been demonstrated in animal models to reduce atherosclerosis through a p53-dependent ATM signaling pathway. Similarly, pioglitazone, a peroxisome proliferator-activated receptor agonist, can increase telomerase activity, TRF-2 expression, and phosphorylation of Akt and reduce the expression of the senescence markers p16, cell-cycle checkpoint kinase 2, and p53.

Currently, it is debatable whether telomere length and telomerase are targets in atherosclerosis. As described above, although atherosclerosis is associated with telomere shortening in multiple cell types, apart from VSMCs there is a paucity of evidence to demonstrate that shortening occurs to critical lengths that impair function. There is also only a little evidence to show that the telomere shortening in vivo makes leukocytes proatherosclerotic. Furthermore, there is minimal evidence to suggest that telomere shortening initiates atherosclerosis rather than being a feature of advanced plaques. Although the lifespan of VSMCs and ECs can be extended by ectopic expression of telomerase, it is not clear if this is due to effects only on telomere length, and the fact that plaque VSMCs cannot be extended indefinitely indicates that plaque VSMCs have other causes for their senescence. In addition, as described above, the mouse studies of telomerase manipulation in atherosclerosis are contradictory. Finally, and most importantly, increasing telomerase expression systemically has considerable risks. Agents that inhibit telomerase are being developed for cancer, indicating that augmenting telomerase has the potential to be carcinogenic and can add the undesirability of uncontrolled proliferation of VSMCs. Therefore, much more research must be undertaken in this area before manipulation of telomere length or telomerase activity as a primary mode of action could be considered for therapeutics in atherosclerosis, although agents that indirectly promote telomere stability in cells on the vessel wall may still be beneficial.

In addition to more specific mechanisms, a number of currently available drugs and compounds are likely to delay premature aging as part of their mechanisms of action through changes in ROS and oxidative DNA damage. Examples of these include antioxidants, statins, and angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARBs). Dietary manipulation and augmentation of sirtuin activity also have potential to reduce vascular aging, cellular senescence, and atherosclerosis.

**Antioxidants**

Expression of naturally occurring antioxidants including fer-ritin and glutathione are reduced with age and in atherosclerosis. Coupled with evidence of increased ROS in atherosclerosis and the demonstration that ROS promote premature aging, this has resulted in intensive study of the use of antioxidants to prevent cardiovascular diseases and aging. However, although antioxidants have been demonstrated to have significant antiatherosclerosis effects in vitro and in animal models, their efficacy in humans is questionable. For example, Vitamin E can reduce the uptake of oxLDL by macrophages and inhibit the subsequent inflammatory responses that stimulate atherosclerosis development. Also, antioxidants can modulate epigenetic patterns through regulating levels of methyl donors and methyltransferase inhibitors. However, there is now extensive evidence indicating that supplementing dietary antioxidants has no significant effect on reducing cardiovascular risk.
Although there may be many reasons why antioxidants might not reduce atherosclerosis, the therapeutic failure of dietary supplementation has simulated interest in enhancing natural antioxidant pathways. For example, overexpression of nuclear factor E2–related factor-2 (Nrf2), a key transcription factor orchestrating antioxidant and cytoprotective responses, in VSMCs and ECs reduces oxidative stress and attenuates inflammatory responses.\(^\text{157,158}\) Nrf2 activators such as Pro-tandim can increase the production of multiple antioxidant enzymes.\(^\text{159}\) In contrast, Nrf2 knockout mice develop smaller atherosclerotic plaques with improved arterial stiffness, regardless of increased serum cholesterol and lipid oxidation.\(^\text{160}\) This phenotype is achieved, at least in part, through down-regulation of the CD36 receptor required for oxLDL uptake by macrophages, which reduces macrophage apoptosis and the subsequent inflammatory responses. These findings demonstrate that Nrf2 mediates multiple pathways independent of its antioxidant effects, and more studies are required before using Nrf2 as a therapeutic option for atherosclerosis.

**Statins**

The hydroxy-methylglutaryl-coenzyme A reductase inhibitors (statins) reduce cholesterol synthesis and hence reduce atherogenesis, the progression of established plaques, and cardiovascular risk. Although their major effect may be via cholesterol reduction, statins have multiple effects that are not necessarily associated with their primary mechanism, including improved endothelial function and reduced inflammation responses. More specifically, statins target mechanisms inducing premature aging, leading to enhanced telomere protection through upregulating TRF2,\(^\text{161}\) decreased DNA damage by accelerating DNA damage repair,\(^\text{61,162}\) and suppressing oxidative stress in part by increasing antioxidant defenses.\(^\text{163}\) Statins can also delay VSMC replicative senescence and reduce markers of DNA damage in vivo in atherosclerosis.\(^\text{61}\)

**ACE Inhibitors/ARBs**

Angiotensin II increases ROS and promotes oxidative DNA damage, promoting senescence through telomeric and non-telomeric DNA damage.\(^\text{82}\) Whereas ACE inhibitors/ARBs have multiple cardiovascular effects, they can also reduce oxidative stress and subsequent DNA damage. At present, it is not known whether these drugs reduce premature aging in vivo and whether this is an important mode of their action. However, bradykinin, a hormone that mediates some of the vasoprotective effects of ACE inhibitors, protects ECs from superoxide-induced senescence through bradykinin B2 receptor-mediated and NO-mediated inhibition of DNA damage.\(^\text{164}\)

**Dietary Manipulation and Sirtuins**

Nutritional status and diet are associated with age-related vascular changes and with atherosclerosis.\(^\text{165}\) In particular, caloric restriction (CR) is a common method to manipulate diet and the beneficial effects of CR on vessel aging have been proven in animal models,\(^\text{166–168}\) including preservation of matrix components within the vessel wall,\(^\text{169}\) improving EC function through augmenting NO generation,\(^\text{170}\) reducing sensitivity to oxLDL,\(^\text{171}\) reducing oxidative stress by upregulating antioxidants and protecting mitochondria function,\(^\text{170,172}\) and inhibiting inflammation.\(^\text{173}\) Although CR may be inappropriate in many patients, drugs and dietary supplements that mimic CR effects without affecting nutritional balance may offer a wider therapeutic option.

Sirtuins (SIRT1–7) are a family of nicotinamide adenine dinucleotide (NAD)\(^+\)-dependent deacetylases and adenoxide diphosphate–ribosyltransferases that may be partially responsible for the age-delaying effects of CR. Sirtuins have been reviewed as part of this series,\(^\text{174}\) therefore only limited discussion is provided here, focusing on SIRT1, the most well-studied member. CR increases SIRT1 in some experimental models, leading to improved endothelial function\(^\text{167,168}\) while knocking down SIRT1 interferes with the CR-mediated antioxidant and anti-inflammatory vascular effects.\(^\text{175}\) Similar to CR, overexpression of SIRT1 in the endothelium can improve vascular stiffness and attenuate the development of atherosclerosis,\(^\text{175}\) probably by activating endothelial nitric oxide synthase (eNOS) and promoting NO production\(^\text{175,176}\) and preventing EC senescence.\(^\text{177}\) Indeed, SIRT deacetylation of eNOS may contribute to the atheroprotective effects of laminar stress.\(^\text{178}\) SIRT1 in hematopoietic cells also prevents foam cell formation and reduces atherosclerosis.\(^\text{179}\) The agent Resveratrol, which can increase SIRT1 expression, reduces EC apoptosis and increases aortic elasticity in aged rodents,\(^\text{180}\) although how much of this effect is due to SIRT1 is unclear. Moreover, other sirtuin members, such as SIRT3–5, as sensors of nutritional status may be protective in vascular system, regulating the cellular response to stress, energy production, apoptosis, and ROS production.\(^\text{181,182}\)

**Conclusions**

In summary, both normal vascular aging and atherosclerosis are associated with cellular senescence. Cellular senescence impairs cell proliferation resulting in irreversible growth arrest and impairs survival, due to an accumulation of nuclear and mitochondrial DNA damage, increased ROS, and a proinflammatory state. Both vascular aging and cellular senescence are associated with increased expression of proinflammatory cytokines and adhesion molecules further promoting inflammation and also affect the synthesis and maintenance of extracellular matrix proteins. Aging can be identified by both structural changes and by a number of senescence-associated biomarkers. However, major gaps in our knowledge exist as to whether small changes in these biomarkers reflect an important loss of function and how aged cells promote diseases. As advanced atherosclerosis is likely to manifest irreversible changes, prevention of accelerated cell aging becomes a major therapeutic opportunity. Understanding the mechanisms contributing to such changes is therefore crucial for both the prevention and the development of treatment for atherosclerosis and other age-related diseases.

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