One of the leading global issues related to human health is age-related diseases, which are of particular concern in developed countries. Aging leads to the progressive decline of normal physiological functioning in all organisms. However, aging or senescence is a complicated biological and social phenomenon that has several multifaceted aspects. In addition, it is difficult to be adequately defined because the physiological defects associated with aging are unique to each individual. Herein, we define aging and senescence as a decline in normal physiological functioning, including the robustness and flexibility observed in mature organisms over time. In particular, aged cells exhibit an increased propensity toward cell death via apoptosis and necrosis, eventually leading to organ failure or defective cellular functioning. Moreover, aging and senescence at a molecular level result in the accumulation of reactive oxygen species (ROS) generated from dysfunctional mitochondria that leads to DNA damage and increases oxidized proteins. Telomere attrition is another well-known mechanism by which cells progress toward senescence.

Cardiovascular aging or age-associated cardiovascular diseases include atherosclerosis, coronary artery disease, hypertension, heart failure, and atrial fibrillation. In addition, aged hearts are characterized by decreased contractility, impaired diastolic function, and atrium dilatation, indicating that both the functional and the morphological alterations that result in these diseases of the heart occur during the aging process. Aged vasculature exhibits morphological changes, including cellular capacity for proliferation or regeneration. In particular, aged cells exhibit an increased propensity toward cell death via apoptosis and necrosis, eventually leading to organ failure or defective cellular functioning. Moreover, aging and senescence at a molecular level result in the accumulation of reactive oxygen species (ROS) generated from dysfunctional mitochondria that leads to DNA damage and increases oxidized proteins. Telomere attrition is another well-known mechanism by which cells progress toward senescence.

Macromolecular Degradation Systems and Cardiovascular Aging

Hiroyuki Nakayama, Kazuhiko Nishida, Kinya Otsu

Abstract: Aging-related cardiovascular diseases are a rapidly increasing problem worldwide. Cardiac aging demonstrates progressive decline of diastolic dysfunction of ventricle and increase in ventricular and arterial stiffness accompanied by increased fibrosis stimulated by angiotensin II and proinflammatory cytokines. Reactive oxygen species and multiple signaling pathways on cellular senescence play major roles in the process. Aging is also associated with an alteration in steady state of macromolecular dynamics including a dysfunction of protein synthesis and degradation. Currently, impaired macromolecular degradation is considered to be closely related to enhanced inflammation and be involved in the process and mechanism of cardiac aging. Herein, we review the role and mechanisms of the degradation system of intracellular macromolecules in the process and pathophysiology of cardiovascular aging. (Circ Res. 2016;118:1577-1592. DOI: 10.1161/CIRCRESAHA.115.307495.)

Key Words: aging • autophagy • inflammation • proteasome endopeptidase complex • ubiquitin
Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5′-UTR</td>
<td>5′-untranslated region</td>
</tr>
<tr>
<td>Ang II</td>
<td>angiotensin II</td>
</tr>
<tr>
<td>ARE</td>
<td>AU-rich elements</td>
</tr>
<tr>
<td>CMA</td>
<td>chaperone-mediated autophagy</td>
</tr>
<tr>
<td>DAMPs</td>
<td>damage-associated molecular patterns</td>
</tr>
<tr>
<td>ECs</td>
<td>endothelial cells</td>
</tr>
<tr>
<td>HuR</td>
<td>Hu antigen R</td>
</tr>
<tr>
<td>IFN</td>
<td>interferon</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>mt-DNA</td>
<td>mitochondrial-DNA</td>
</tr>
<tr>
<td>mTOR</td>
<td>mechanistic target of rapamycin</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor-κB</td>
</tr>
<tr>
<td>NLRP3</td>
<td>NOD-like receptor 3</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>SASP</td>
<td>senescence-associated secretory phenotype</td>
</tr>
<tr>
<td>TLR</td>
<td>toll-like receptor</td>
</tr>
<tr>
<td>TNFα</td>
<td>tumor necrosis factor-α</td>
</tr>
<tr>
<td>UPS</td>
<td>ubiquitin–proteasome system</td>
</tr>
<tr>
<td>VSMCs</td>
<td>vascular smooth muscle cells</td>
</tr>
</tbody>
</table>

calcification and cholesterol-rich plaque formation, as well as defective relaxation and endothelial dysfunction that are rarely observed in youth. Thus, alterations of the heart associated with aging occur in both the vasculature and the myocardium. In the myocardium, impaired contractility associated with cardiac aging or senescence is prompted by an ROS-induced increase of oxidized proteins and lipids in the contractile machinery and altered cellular homeostasis. Mitochondrial dysfunction and dynamics are also closely related to this process.

Notably, a plethora of evidence indicates that the degradation system exemplified by declined or impaired autophagy has a crucial impact on senescence development, including within the cardiovascular system. Herein, we review the role and mechanisms of the degradation system of intracellular macromolecules in the process and pathophysiology of cardiovascular aging.

Mechanisms of Aging and Senescence

There are 3 major mechanistic scenarios in the progression of aging and senescence based on biological and epidemiological evidence from Caenorhabditis elegans as well as humans, namely, free radical theory, programmed cellular senescence, and inflamming.

Reactive Oxygen Species

Oxidative stress causes cell damage by chemical modification, such as the oxidation of proteins, nucleic acids, and lipids. The increase and accumulation of such damage promote aging. The gradual mitochondrial damage and dysfunction accompanied by mitochondrial-DNA (mt-DNA) mutations has been found to accompany aging, and somatic mt-DNA mutations expand clonally to cause mosaic respiratory chain deficiency in different aging organs including heart. The free radical theory of aging is commonly accepted as the central mechanism of cellular senescence, whereas some recent evidence has demonstrated that increased ROS production does not necessarily shorten the lifespan of C. elegans. Among ROS, hydroxyl radicals generated from hydrogen peroxide and iron resulting from the Fenton reaction robustly oxidize proteins, DNA, and lipids. Recently, a more complex mechanism other than macromolecular damage by oxidation has been shown to contribute to cellular aging. This mechanism involves oxidative stress that promotes telomere attrition. The oxidation of guanine in the 5′-TTAGGG-3′ telomere repeat sequence is susceptible to ROS and is involved in the shortening machinery. Moreover, ROS-induced DNA damage can lead to the decline of intracellular NAD+ and decreased sirtuin activity in heart, lung liver and kidney in female rat that promote cellular senescence.

Cellular Senescence and Regulatory Signaling

There are several genes and low molecular weight compounds reported to prolong the lifespan of yeast, C. elegans, Drosophila, and mice, suggesting that conserved signaling mechanisms to regulate aging should exist. At least 3 major signaling pathways have been proposed to regulate cellular senescence in eukaryotes: (1) the insulin/IGF-1 pathway, (2) TSC/mechanistic target of rapamycin (mTOR) pathway, and (3) sirtuins. Moreover, telomere attrition is highly involved in the cellular senescence of proliferative cells. As these issues are intensively described elsewhere in a review series, this will not be discussed here in detail except the TSC/mTOR pathway that is closely related to macromolecular degradation and inflammation-induced aging, termed as inflamming.

The mTOR is a serine/threonine kinase that is highly conserved from yeast to worms, flies, and mammals, including mice and humans. mTOR is the catalytic subunit of protein complexes termed mTOR complex 1 and mTOR complex 2, which regulate nutrient and growth factor signaling downstream of anabolic processes. There are numerous findings demonstrating that mTOR signaling has a negative impact on longevity. In C. elegans, the loss of mTOR signaling increases the mean lifespan by 2.5-fold, and the effect is independent of the forkhead box O family of transcription factors related to the insulin/IGF-1 signaling pathway. Moreover, the loss-of-function assays for multiple types of substrates downstream of mTOR leads to an extended lifespan in worms, suggesting that it plays a crucial role in cellular senescence. Furthermore, there are several reports that these signaling pathways are involved in longevity in Drosophila and mice.

Inflamming and Senescence-Associated Secretory Phenotype

It is firmly established that aging is associated with alterations in the immune system in humans. In particular, there is a myriad of evidence on age-associated alterations in the development and function of B and T cells. Moreover, the acute inflammatory response against pathogens declines during the aging process and confers increased susceptibility to infections. In contrast, there is a chronic low-grade increase in the levels of steady-state serum proinflammatory cytokine in the majority of elderly individuals. These results indicate that chronic inflammation is induced and sustained along with aging (inflamming). In addition, aged cells including
fibroblasts represent a characteristics known as the senescence-associated secretory phenotype (SASP), namely, production of the secretome of cytokines, growth factors, extracellular matrix proteins, and proteases.12 These secreted factors include typical proinflammatory cytokines, such as interleukin (IL)-1β, IL-6, and IL-8, suggesting that chronic inflammation is strongly related to pathological senescence. The mechanism underlying elevated basal inflammation associated with aging remains incompletely understood. One proposed mechanism of inammaging is the chronic stimulation of immune cells by a persistent cytomegalovirus infection.30 On the contrary, the alteration in innate immune functionality is thought to be involved in the development of age-associated chronic inflammation. Indeed, SASP acquisition was observed in immune cells and in fibroblasts and endothelial cells (ECs).31 Numerous changes in innate immunity are considered to involve multiple innate immune cells, including the altered expression of pattern recognition receptors, and pattern recognition receptor activation by endogenous ligands derived from damaged cells.32 Damage-associated molecular patterns (DAMPs) released from senescent or dead cells, including cholesterol, ceramide, ATP, heparan sulfate, and endogenous nucleic acids, can evoke chronic inflammation associated with aging.33 Along with aging, DAMPs can increase and become involved in the underlying mechanism of SASP expression. In addition, the increase in post-translationally modified proteins or oxidized DNA can potentially induce the upregulation of basal inflammation.34 It has been established that oxidative stress itself promotes inflammation in a toll-like receptor (TLR)- or NOD-like receptor 3 (NLRP3)-dependent manner.35 Indeed, the activation of an innate immune response can be elicited via TLR2 and TLR4 by oxidized lipoproteins in human macrophages.35 On the contrary, NLRP3 inflammasomes are directly activated by sustained ROS stimulation in T-helper cell 1.36 Aging is associated with multiple TLR expression in innate immune cells. However, there is an age-associated decrease in TLR1, TLR3, and TLR8 expression observed in dendritic cells.37 Similarly, there is a decline in the expression of TLR7 and TLR9 in plasmacytoid dendritic cells.38

Nonagenarian women of 91 to 92 years of age exhibit higher plasma circulating cell-free DNA (one of DAMPs) levels than younger controls.39 Moreover, in a nonagenarian cohort, the levels of circulating cell-free DNA are strongly associated with inflammatory markers in the plasma, such as C-reactive protein, and are an independent risk factor of mortality.40 In light of downstream DAMP signaling, the activation of nuclear factor-κB (NF-κB) is significantly higher in old mice over 24 months of age than in younger mice of 3 months of age. The activation of NF-κB signaling via DNA damage could be critical in the observed enhanced production of proinflammatory cytokines by SASP.41 Thus, the accumulation of DNA damage accelerated by oxidative stress could be one of the leading causes of the basal inflammation that exists in aging individuals.

mt-DNA also can be released into the extracellular space from damaged or dead cells and can lead to the inammation by acting as DAMP.42 Circulating mt-DNA levels increase with age and are positively correlated with the plasma levels of proinflammatory cytokines, such as tumor necrosis factor-α (TNFα) and IL-6 in human.43 In addition, we previously reported that intracellular nondegraded mt-DNA induces the innate immune response leading to inflammation via TLR9-dependent signaling in mouse hearts.44 Thus, it is possible that the increased, undigested mt-DNA associated with aging is involved in SASP development in an autonomous manner. An enhanced generation of saturated fatty acids and ceramides leads to the activation of the NLRP3 inflammasomes, resulting in insulin resistance and lipotoxicity of metabolic syndrome.45

It has been shown that Dicer expression, which plays a central role in micro-RNA (mi-RNA) processing, is decreased in the adipose tissue of aged mice or preadipocytes from aged humans.46 Genetically engineered mice lacking Dicer in the adipose tissue exhibit increased sensitivity to oxidative stress and senescent phenotypes, suggesting that the dysregulation of RNA degradation by mi-RNA is involved in the development of the age-associated proinflammatory environment.46 Taken together, the degradation system declines in aging process, and the overflow of unprocessed macromolecules, such as oxidized proteins or nucleic acids, could lead to the activation of innate immunity (Figure 1).

Macromolecular Degradation

Degradation of Proteins

There are 2 forms of protein degradation: intracellular and extracellular degradation. Extracellular degradation of proteins involves dissolution by digestive enzymes in the alimentary canal and is primarily nonselective. We will discuss intracellular protein degradation in this article. Three forms of intracellular protein degradation include the following: (1) selective degradation by specific proteases, (2) broader spectrum of protein degradation by the ubiquitin/proteasome system, and (3) autophagy. The latter 2 processes result in complete protein degradation to small peptides or individual amino acids, whereas selective proteases mainly induce cleavage to alter or abrogate protein function.

Cysteine Proteases

Proteases include exopeptidases that cleave 1 or multiple amino acids at the N or C terminus, and endopeptidases that cleave internal peptide bonds.47 Endopeptidases are classified into aspartic, serine, threonine, metallo, and cysteine proteases based on their catalytic mechanism.47 Among the intracellular proteases, cysteine proteases, including calpains, cathepsins, and caspsases, are well known and characterized by structure and biological function. Those proteases that induce protein cleavage rather than degradation thereby lead to loss, alteration, or activation of substrates.

Calpains are Ca2+-dependent cysteine proteases that cleave intracellular substrates, such as cytoskeletal proteins, kinases, phosphatases, membrane-associated proteins, and transcription factors.48 In addition, they are involved in a myriad of biological functions, including cell migration, differentiation, apoptosis, and membrane repair.49,50 There are 15 calpain gene family members in mammals, of which μ- and m-calpains have been widely studied.50 Calpain activation is a tightly regulated process to prevent deleterious consequences of massive...
proteolytic activity. These regulatory mechanisms seem to decline with aging, resulting in increased calpain activity.

Caspases consist of a cysteine protease cascade that is well defined as an essential proteolytic pathway to induce apoptosis. The dysregulation of apoptosis has been shown to be involved in age-related brain dysfunction, such as Alzheimer disease. In addition to apoptosis, increasing evidence indicates that multiple functions of caspase-mediated protein degradation, including tissue differentiation, regeneration, neural development, genome stability maintenance, autophagy, and inflammation. Notably, caspase-1 plays a crucial role in NLRP3 inflammasome activation by cleaving pro–IL-1β or pro–IL-18 for maturation to induce systemic inflammatory responses that are possibly related to age-related inflammation.

Cathepsins are a family of cysteine proteases containing at least 12 cathepsins that share a conserved active site formed by cysteine, histidine, and asparagine residues. Of these, cathepsin B and L have been well studied with respect to physiological and pathological functions. Cysteine cathepsins are synthesized as inactive precursors and are usually activated in the acidic environment of the lysosomes. Thus, they were initially considered to be intracellular enzymes that functioned in the acidic environment of the endosomal and lysosomal compartments for nonspecific, bulk proteolysis. Recently, important and specific functions of cathepsins have been discovered that occur extracellularly and in other locations, such as secretory vesicles, the cytosol, and the nucleus, and are involved in the proteolytic processing of specific substrates.

Ubiquitin–Proteasome System

The ubiquitin–proteasome system (UPS) is the main ATP-dependent protein degradation machinery in the cytosol and nucleus of eukaryotic cells and is responsible for the degradation of 80% to 90% of intracellular proteins. Protein degradation via the ubiquitin–proteasome pathway involves 2 discrete and successive steps. In the first, the substrate is tagged by a covalent attachment of multiple ubiquitin molecules (8.5 kDa as a monomer) by E1-3 ubiquitin enzymes. This is then followed by the second step that involves the degradation of the tagged protein by the 26S proteasome complex with the release of free and reusable ubiquitin. The covalent modification of target proteins by ubiquitin involves an enzymatic cascade that includes an E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme, and E3 ubiquitin ligase, which recruits protein substrates and mediates ubiquitin attachment. The 26S proteasome is a 2.5-MDa complex consisting of a pair of components containing at least 32 different subunits that are highly conserved among all eukaryotes. The overall structure of 26S proteasome can be divided into 2 major subcomplexes: (1) 20S containing the protease subunits and (2) 19S regulatory complex (Figure 2).
In addition to the central role in protein degradation to remove oxidized or damaged molecules, UPS plays a significant role in intracellular signaling regulation, including NF-κB signaling, AMP-activated protein kinase activity, cyclin-dependent kinase and inhibitors, and forkhead box O-dependent transcription via protein processing. Through the processing of those proteins, UPS contributes to the regulation of multiple biological functions, including immune and inflammatory responses, cellular metabolism, autophagy, cell proliferation, and possibly senescence.

There are several reports that an alteration of proteasome in senescence and regulation of lifespan. Proteasome has been reported to play major roles in protein turnover and oxidized protein removal, one of the main triggers for the development of cellular senescence. In addition, the over-expression of proteasome subunits has been shown to extend the lifespan of C. elegans and yeast. Proteasome levels and function decline with advancing age in various mammalian tissues, including those in humans. A dysfunctional proteasome is related to numerous age-related diseases both in humans and animal models, suggesting that the removal of damaged or oxidized proteins by the UPS system is indispensable for the prevention of cellular senescence. Recently, an alternative form of the 20S proteasome, also known as immunoproteasome, has been reported to correlate with lifespan in mice and primates. Immune proteasome contains alternative forms of proteolytic subunits, including proteasome subunit β8, proteasome subunit 9, and proteasome subunit 10,
and is upregulated under oxidative stress in cultured cells (Figure 2). Thus, immunoproteasome plays an important role in the degradation of oxidized proteins. On the contrary, oxidized protein is also degraded by regular 20S proteasome in an ubiquitin-independent manner. However, extensive ROS cause post-translational modification of subunit and

Figure 3. Degradation of DNA and inflammation. Degradation of superfluous DNA by three DNase (DNase I, II, and III) prevents sterile inflammation. The secreted extracellular DNase I degrades inflammogenic genomic DNA released by necrotic cells, what is called a damage-associated molecular patterns, to prevent inflammasome activation. The lysosomal DNase II degrades mitochondrial-DNA during autophagic process thereby hamper toll-like receptor 9-dependent immune responses. Intrinsic self-DNA is degraded by DNase III/3′ repair exonuclease 1 in cytosol, whereby hinder stimulator of interferon gene (STING)–dependent induction of cytokine production caused by cytosolic DNA.

and is upregulated under oxidative stress in cultured cells (Figure 2). Thus, immunoproteasome plays an important role in the degradation of oxidized proteins. On the contrary, oxidized protein is also degraded by regular 20S proteasome in an ubiquitin-independent manner. However, extensive ROS cause post-translational modification of subunit and

Figure 4. Regulation of AU-rich element (ARE)–mediated mRNA decay by RNA-binding proteins. The exemplified model of regulating inflammation by mRNA decay with specific RNA binding proteins is shown. Tristetraprolin (TTP) promotes ARE-mediated mRNA decay of TNFα thereby prevent sustained inflammation. Hu antigen R (HuR) counteracts TTP and stabilizes ARE-containing mRNAs including TNFα thereby culminate in long-lasting inflammation.
elicited oxidized protein aggregation both which may inhibit ubiquitin-dependent/ubiquitin-independent proteasomal activity in advanced aging.\textsuperscript{75,76}

**Autophagy**

Autophagy is a genetically programmed, evolutionarily conserved catabolic process that degrades cellular proteins and damaged or excessive organelles via their engulfment by a double-membrane structure termed autophagosome.\textsuperscript{77} There are 3 main autophagic pathways: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA). Macroautophagy is the most prevalent form and commonly referred to as autophagy. Both autophagy and CMA are important pathways involved in protein degradation and can be activated under the stressed conditions. When CMA is inhibited, autophagy is upregulated. CMA compensates when autophagy is inhibited.\textsuperscript{78} However, age-related changes in the lipid constituents of lysosomal membrane lead to both autophagy and CMA activity decrease with age in most organs in aging mammals.\textsuperscript{79} There are multiple comprehensive reviews on autophagy and its role in the heart.\textsuperscript{77,80,81} The autophagy in cardiac aging will be discussed elsewhere in this review series.

**Degradation of Nucleic Acids**

There are 2 types of DNA degradation as follows: (1) the complete degradation of DNA from a microorganism or mitochondria via DNase, including DNase I and II and (2) a restricted area of nucleotides caused by exonucleases that contribute to genome stability, including DNA repair. DNase I is a Ca\textsuperscript{2+} and Mg\textsuperscript{2+}/Mn\textsuperscript{2+}-dependent secretory exonuclease and hydrolyzes double-stranded DNA. Extracellular DNase I participates in the chromatin breakdown of necrotic cells or microorganisms\textsuperscript{82} (Figure 3). DNase II is an acidic enzyme localized in the lysosome and hydrolyzes the phosphodiester backbone of DNA molecules by a single-stranded cleavage mechanism.\textsuperscript{83} In addition, DNase II exhibits an acid pH optimum of \(\approx 5.0\), thereby exhibiting suitable function to degrade any DNA inside the lysosome\textsuperscript{83} (Figure 3). DNase III, also known as 3' repair exonuclease 1, is a 3' to 5' exonuclease with a preference for single-stranded DNA or mispaired 3' termini and is expressed in most mammalian cells.\textsuperscript{84} DNase III/3' repair exonuclease 1 catalyzes single-stranded DNA polynucleotides derived from the processing of aberrant replication intermediates to attenuate DNA damage-induced checkpoint activation.\textsuperscript{85} In addition, DNase III/3' repair exonuclease 1 degrades cytosolic self-DNA to prevent activation of innate immune sensor stimulator of interferon genes, an endoplasmic reticulum-associated transmembrane-rich molecule that is activated by cytosolic DNA to induce production of multiple cytokines\textsuperscript{86} (Figure 3).

RNA steady-state maintenance is the result of the synthesis and degradation of transcripts. mRNA metabolism in the nucleus includes capping, splicing, and polyadenylation as these processes are mechanistically linked to transcription process.\textsuperscript{87} There are 2 major cytoplasmic pathways for mRNA degradation that begin with the shortening of the poly(A) tail by deadenylase complexes.\textsuperscript{88} After deadenylation, mRNA can be degraded from the 3' end by exosome, resulting in an oligonucleotide with a cap that has been removed by a salvage pathway catalyzed by Dcs1.\textsuperscript{89} Alternatively, decapping via the Dcp complex leads to mRNA decay caused by a 5' to 3'exoribonuclease Xrn1, which contains an RNA binding and RNase catalytic domain in its N-terminal region.\textsuperscript{90} mRNA decay may be regulated by a transcriptional program that responds to extracellular stress. The transcriptional and post-transcriptional responses allow a more rapid adaptation to new environmental conditions than any other mechanism acting alone. The regulation of mRNA decay is frequently mediated by sequence-specific RNA-binding proteins. Gene or class-specific RNA-binding proteins regulate RNA decay by binding sequences in either the 5'-untranslated region, open reading frame, or more commonly 3'-untranslated region.\textsuperscript{91} AU-rich elements (ARE) localized in 3'-untranslated region are the best-characterized cis elements. Indeed, ARE-binding proteins, including tristetraprolin and Hu antigen R (HuR), regulate mRNA stability via the ARE-mediated pathway. Tristetraprolin destabilizes cytokine-encoding mRNA, such as TNF\alpha, by binding to AREs on mRNA and promoting deadenylation by exonucleases. In contrast, HuR stabilizes ARE-containing mRNAs, such as TNF\alpha, cyclin A, and cyclin B1 (Figure 4). In addition, mRNA decay of IL-6 regulated by Regnase-1 directly inhibits innate immune responses.\textsuperscript{92} mi-RNAs regulate gene expression at the post-transcriptional level via target mRNA degradation and translational suppression via binding 3'-untranslated region.\textsuperscript{93}

**Macromolecular Degradation and Aging in the Vasculature**

**Aging in the Vasculature**

Characteristic features of the large and small arteries associated with aging include endothelial dysfunction, vascular remodeling, plaque formation, inflammation, calcification, and increased stiffness of the vascular walls.\textsuperscript{8} These alterations are similar to those observed in younger patients with hypertension, suggesting that common signaling or molecular mechanisms exist for these pathological changes. In the aged vasculature, the fragmentation and calcification of elastic fibers are observed and an increased deposition of collagen, collagen cross-linking, amyloid deposition in the intermediate layer, and proliferation of vascular smooth muscle cells (VSMCs).\textsuperscript{9} These alterations are mediated by oxidative stress and proinflammatory cytokine secretion.\textsuperscript{94} In human coronary arteries, ECs with \(\beta\)-galactosidase activity associated with enhanced senescence are observed during aging.\textsuperscript{95} This suggests that cellular senescence is involved in atherosclerosis development.

Cellular senescence is thought to be a state of irreversible cell cycle arrest accompanied by alterations in gene expression, including increased proinflammatory cytokine production and cellular morphology.\textsuperscript{1} Similar to cellular senescence in other cell types, oxidative stress and inflammation play major roles in promoting senescence in the vasculature. For instance, vascular calcification, a typical phenotype of aged vasculature, is prompted by DNA damage that induces secretion of IL-6 in prelamin A–positive VSMC.\textsuperscript{96} In addition,
proinflammatory cytokine levels are elevated in the serum of elderly patients with no overt disease.97 Moreover, a recent study displayed that senescent human VSMCs develop a proinflammatory state known as SASP.98 These results suggest that inflammation associated with the normal aging process contributes to the onset of age-related diseases. Ang II stimulation is one of the factors responsible for the cellular senescence of the vasculature.99 Interestingly, Ang II is the one of the most dominant signaling pathways in pathogenesis of hypertension. Indeed, the blockade of both the angiotensin-converting enzyme and the Ang II type 1 receptor is the first choice of treatment for patients with hypertension.100 Ang II stimulation induces pathological changes similar to those associated with aging in the vasculature.99 In addition, Ang II can induce an inflammatory response, and Ang II and cytokines may synergistically promote vascular aging.101 Consistently, a disruption of Ang II type 1 receptor in mice exhibits a marked increase in the lifespan with less vascular injury and oxidative damage.102 Thus, renin–angiotensin system signaling plays a dominant role in the development of vascular aging.

**Cysteine Protease and Aging**

Ang II induces calpain-1 expression and activity in the arterial wall and within the human aortic intima in the aged.103 Calpain-1 activation leads to enhanced matrix metalloproteinase 2 activity, resulting in collagen I and III production and arterial calcification.104 Moreover, pharmacological calpain inhibition attenuates the aging phenotype, including artery calcification in a murine model of α-klotho deleted progeria.104 Taken together, increased protein degradation by calpain contributes to vascular aging in mice and humans.

As described above, caspase-1 plays a major role in NLRP3 inflammasome activation via the cleavage of pro–IL-1β and IL-18.55 Inflammasome activation enhances calcification in human VSMCs.105 In addition, a recent report indicates that inflammasome inhibition prevents calcification in cultured VSMCs.106 Cytokine production by inflammasome activation in VSMCs likely plays a significant role in the calcification associated with type 2 diabetes mellitus, an age-related disease associated with a proinflammatory state.107 Glucose induces inflammation in VSMCs108 and activates the inflammasome-dependent release of IL-1β, which stimulates VSMCs calcification. In mice, the deletion of NLRP3 that lacks the inflammasome-dependent release of IL-1β exhibits improved glucose tolerance and insulin sensitivity, which are important pathogenesis in type 2 diabetes mellitus.108 Caspase-1 inhibition increases insulin sensitivity, suggesting a central role in the development of vascular calcification.109

The expression of cysteine cathepsins S, K, and L is enhanced in human atherosclerotic lesions.110 In addition, atherosclerosis-associated inflammatory cytokines augment cysteine cathepsin expression and activity in VSMCs, indicating a pathological role of cathepsins in vascular aging.110 Moreover, stress-induced premature senescence of ECs has emerged as a contributor to global EC dysfunction and thought to be involved in age-related disease.111 Sirtuin 1 contributes to this phenotype because the cysteine cathepsins B, S, and L directly cleave sirtuin 1 and mediate stress-induced premature senescence.112

**UPS and Aging**

There are controversies on the proteasome activity in atherosclerosis. Several previous reports have demonstrated a consistent increase in ubiquitin conjugates in human coronary, carotid, and cerebral arteries113,114 in middle-aged individuals. Other studies have noted an increase in proteasome activity in high-risk plaques and even in macrophages extracted from these plaques.115 However, other evidence indicates that proteasome activity declines with age and is lower in atherosclerotic plaques of elderly patients.116 Taken together, although the activity of UPS is upregulated in early developing or progressing atherosclerosis, a decrease in proteasome function contributes to vascular aging, including atherosclerosis.

A study using a comprehensive omics approach identified UPS as a major system that leads to alterations of multiple biological processes, including inflammation, proliferation, and angiogenesis.117 Notably, immunoproteasome activation via the induction of proteasome subunit β is identified as a potential link between inflammation and apoptosis of atherosclerotic plaques.118 However, the mechanism by which this occurs is needed to be determined in future research. Mechanistically, UPS is involved in a myriad of the atherosclerotic processes involving substrate degradation, including the transcription factors nuclear erythroid 2–related factor, hypoxia-inducible factor 1α, and NF-κB.119

**Autophagy and Aging**

During the aging process, it has been reported that the capacity of the synthesis and degradation systems declines.120 With regards to protein degradation, the gradual shift from the proteasome pathway to the autophagic process has been observed during replicative senescence in a human iB90 cell model of aging.121 A myriad of evidence indicates a strong association between senescence and autophagy in various cell types.122 The autophagic activity demonstrates a decrease in multiple organs, including the heart, liver, and hypothalamus associated with the aging process in mice.122,123 Therefore, the inhibition or induction of autophagy facilitates or delays senescence, respectively.11 With increasing age, vascular autophagy seems to decline, and this decline may contribute to the impairment in endothelial function.124 Ang II induces autophagy in VSMCs, as well as ECs, and Ang II stimulation leads to an impairment of mitochondrial function, thereby increasing oxidative stress, which eventually aggravates cellular functions.124 Because autophagy is a protective effect observed in many organs, including the heart,80 Ang II–induced autophagic activity in VSMCs could be an adaptive response to neurohumoral stress. In ECs, autophagy possibly prevents senescence and apoptosis evoked by Ang II stimulation.125 Pharmacological agents, such as resveratrol, stimulate autophagy and delay many aspects of vascular aging.124 Thus, autophagic degradation could play a protective role in pathological aging. However, the functional role of macromolecular degradation via methods other than autophagy remains elusive.
Degradation of Nucleic Acids and Aging

Nucleic acids in the serum, a major molecule of DAMPs, can bind to TLRs and elicit inflammation.33 About vascular inflammation, high mobility group protein B1, an endogenous TLR9 ligand, elicits inflammation and lesion formation via TLR9 activation in a mouse vascular injury model.126 Because TLR9 is a sensor for endogenous DNA, including mt-DNA, undegraded DNA circulating in the serum could bind to TLR9 expressed on vascular cells and may contribute to vascular inflammation, eventually resulting in vascular aging.

Relatively few studies have examined the functional relationship between mRNA decay and vascular aging. HuR is a regulator of the cell cycle, which functions at least in part by mediating cell cycle–dependent stabilization of mRNAs encoding cyclins A and B1.127 HuR can also stabilize mRNAs of sirtuin 1 and vascular endothelial growth factor.128 HuR expression is decreased in senescent cells, and its target mRNAs encoding cyclin A, cyclin B1, and c-fos also decrease with replicative senescence.129 The cells overexpressing HuR exhibit features of young cells. HuR expression is also decreased in the aorta of aged rats,130 suggesting that HuR is involved in vascular aging. Tristetraprolin can destabilize TNFα and vascular endothelial growth factor mRNAs.131 Tristetraprolin induces senescence in human papillomavirus-transformed cervical cancer cells by targeting E6-AP ubiquitin ligase.132 However, it remains unknown whether tristetraprolin is involved in vascular aging. In contrast, some miRNA have been associated with vascular aging.32 miR-34a induces senescence of ECs and VSMCs. miR-217 induces EC senescence and dysfunction, whereas miR-146a inhibits EC senescence.93 miR-29 also induces aortic dilatation and aneurysms.

Macromolecular Degradation and Aging in the Myocardium

Aging in the Myocardium

The heart, as a pumping organ, ages via 2 different processes: physiological and pathological aging. Pathological aging refers to a dysfunctional state out of deviation from average state of correspondingly aged person. The aged myocardium involves several pathophysiological characteristics, including increased thickness of the left ventricular wall, decreased cardiac reserves, and decreased responsiveness to β-adrenergic stimulation.3 In addition, the enlarged mitochondria characterized by swelling, cristae loss, and matrix deformities are important aspects of the age-related changes exhibited by cardiomycocytes.131 The senescent mitochondria also exhibit decreased ATP production and increased ROS generation. Over time, cardiomycocytes accumulate large amounts of lipofuscin, which are composed of lipid-containing residues of lysosomal digestion and a hallmark of aging.134 All of these features are closely related to a decreased adaptation to stress or mechanical overload.

The heart comprises multiple types of cells, including cardiomyocytes, cardiac fibroblasts, VSMCs, ECs, and cardiac stem cells. Myocardium aging may be construed as a complex of cellular senescence in multiple individual cell types. Notably, each cell type might represent a distinct form of aging. For instance, cardiomyocytes are considered to have a low potential for proliferation in physiological condition, and the contribution of telomere attrition to cellular senescence of these terminally differentiated cells remains unknown. Furthermore, telomere length in patients with dilated cardiomyopathy is 25% shorter than that in healthy controls.135 In addition, telomere attrition is also observed in elderly patients with heart failure.136 In mice, an age-induced expression of miR-34a elicits telomere shortening, and the genetic deletion of miR-34a results in decreased cardiac aging.137 On the contrary, multiple signaling pathways that play significant roles in cellular senescence are involved in the development of cardiac hypertrophy or cell death and are prominent features observed in aged hearts.

Experimental Models of Aging

Experiments using rodent models to analyze the underlying mechanism of cardiac aging involve the critical problem in extrapolating the results to humans, given the significantly shorter lifespan of other species than of primates. Despite this limitation, multiple experimental models, including aging, Ang II stimulation, and pressure overload are thought to be useful in investigating the mechanism and implication in cardiac senescence.3,2

Although the chronic stimulation of Ang II in mice is a well-known model of cardiac hypertrophy, recent evidence suggests that Ang II is a strong inducer of cellular senescence in several cell types other than cardiomycocytes. Moreover, it can also mediate the proinflammatory profile observed in an aged heart.138 Furthermore, in rodents, chronic alterations in the structure and function of the heart seen with advancing age are similar to experimental left ventricular pressure-overload model in light of histological, physiological, and biochemical features.2 This includes increased cell size, fibrosis, prolongation of action potential and Ca2+ transient durations, declined sarcoplasmic reticulum Ca2+ pump rate, and alterations of myosin isozyme composition.2 Thus, experimental data from these models could contribute to examining the molecular mechanism of cardiac aging.

Inflammation of the Heart

There are multiple stimulations that can elicit cytokine production in cultured cardiomycocytes. Myocytes produce TNFα in response to lipopolysaccharide stimulation139 or increased IL-6 production after IL-1β administration.140 As previously described, cytokines and Ang II are strong inducers of cellular senescence.4 Notably, Ang II is a neurohumoral factor that is closely related to cellular senescence and provokes cytokine production in cardiomycocytes, which could synergistically induce a phenotypic alteration associated with aging.3 For instance, isolated adult cardiomycocytes prepared from a hypertrophied heart of a rat by pressure overload secrete TNFα, IL-1β, and IL-6 after Ang II administration.141 Moreover, in neonatal rat cardiomycocytes, stretching cells combined with Ang II stimulation induce the upregulation of IL-13, an SASP factor.142 In the left ventricle of senescent rats, the gene expression of angiotensinogen and angiotensin-converting enzyme is enhanced,139 suggesting that Ang II plays an important role in the development of the aged myocardium. Cardiomycocyte growth is affected by the systemic environment and potentially...
by extracardiac growth factors. Neonatal cardiomyocytes implanted in the peritoneal cavity of aged rats exhibit impaired cellular growth from those of younger rats. This implies that aging attenuates the induction of cardiomyocyte growth by systemic factors. Moreover, Ang II stimulation induces proinflammatory cytokine production in cardiac fibroblasts and accelerates IL-6 and TNFα synthesis in cardiomyocytes in a paracrine manner. Intriguingly, mTOR signaling, one of the dominant mechanisms of cellular senescence, plays a major role in such proinflammatory cytokine production or secretion.

Cytokine production might represent a characteristic feature in aged myocytes, similar to the biological phenomenon observed in the cellular senescence of other cell types as SASP. The cytokine expression in cardiomyocytes is analogous to SASP because of the following reasons: (1) it occurs along with aging, (2) oxidative stress plays a crucial role in the development of this biological phenomenon, (3) senescence-related neurohumoral factor Ang II induces the secretion of IL-6 and TNFα in cardiomyocytes, and (4) the Ang II–mediated proinflammatory profile is also observed within the aged heart. Furthermore, the mTOR signaling pathway is reported to be an important senescent signaling pathway in other types of cells and lead to the production of cytokines in cardiomyocytes. Thus, cytokine induction related to sterile inflammation in the aged heart could be SASP. However, this hypothetical scenario is yet to be determined by establishing the molecular mechanism and functional role of mTOR signaling in cardiac aging.

Cysteine Proteases and Aging
Calpain-1 expression levels increase in aged canine atrial myocytes with fibrillation and are involved in electric and structure remodeling. Although calpains modulate the activity of enzymes and induce specific cytoskeletal rearrangements in various aging phenomena and age-related diseases, detailed evidence is lacking, particularly in the ventricles.

Although cardiac apoptosis increases with aging in the myocardium, the frequency of apoptotic cardiac cell death is extremely low. There are previous reports that show caspase-3 activation after a pressure overload or Ang II stimulation, suggesting an important role of caspase activation in myocardium aging. Although some reports have demonstrated inflammasome activation during myocardial infarction in mice, the functional role of caspase-1 in inflammasome activation in aged cardiomyocytes remains unknown. About aging-related neurohumoral factor stimulation, NLRP3-deficient mice are protected against Ang II–induced cardiac fibrosis with preserved cardiac architecture. Moreover, cardiac fibroblasts are the main source of inflammatory cytokines in this genetically engineered model. Therefore, the mechanism of inflammasome activation in aged myocardium needs to be examined.

It has been shown that cathepsin B activity increases with aging in the brain. Although increased expression and activity of cathepsins during the degeneration of the central nervous system are frequently reported, the role of cathepsins in cardiac aging remains to be elucidated, specifically in humans. In mice, cathepsin K deletion alleviates the age-dependent decline in cardiac function. Aged mice exhibit significant cardiac remodeling, including enlarged chamber size, wall thickness, myocyte cross-sectional area, fibrosis, and decreased cardiac contractility along with compromised intracellular Ca²⁺ release compared with young mice, which were attenuated in cathepsin K–deficient mice.

UPS and Aging
Previous study have demonstrated a potential role for an age-related inhibition of a key intracellular protease, proteasome, as a potential mediator of age-related pathogenesis in the heart. Both UPS as well as autophagy and lysosome systems exhibit a decreased efficiency as a consequence of aging. Moreover, a dysfunction of these systems is associated with cardiomyopathies. In human, proteasome activity in both hypertrophic cardiomyopathic and failing hearts is impaired suggesting proteasome functional insufficiency plays a major role in cardiac pathogenesis. In addition, enhancement of proteasomal function causes beneficial effect against cardiac proteinopathy and ischemia/reperfusion in mouse experimental models. The functional role of UPS in the development of cardiac hypertrophy is more controversial. The increase in proteasome expression and activity is found during chronic pressure overload and pharmacological inhibition of proteasome prevents cardiac hypertrophy in a canine experimental model. In contrast, a group reported that decreased proteasome activities were observed during the progression of cardiac dysfunction in pressure-overloaded heart of mice.

The inhibition of mTOR by rapamycin has been shown to attenuate pathological cardiac hypertrophy and improve the function of the aging heart, accompanied by an inhibition of the cardiac proteasome activity. Concurrent with the decrease of immunoproteasome, rapamycin decreases the inflammatory response pathways, such as NF-κB and signal transducers and activator of transcription 3 signaling, suggesting that an alteration in the proteasome is related to inflammation. Thus, the declined UPS may be a promising therapeutic target for cardiac aging.

Autophagy and Aging
Autophagy is an important quality control pathway required to maintain cardiac homeostasis and adapt to stress. We previously reported that the inhibition of macromolecular degradation attenuates the reverse remodeling of Ang II–induced cardiac hypertrophy. Moreover, impaired autophagy leads to cardiac dysfunction after pressure overload, indicating that decreased macromolecular degradation is involved in cardiac dysfunction in cardiac aging of which phenotype includes similar features. A decrease in autophagy has been observed in several aging models. Lipofuscin, one of the aging pigment granules, have been observed and proposed to contribute to the decreased autophagic flux in aging cardiomyocytes by impairing lysosomal function. We reported that continuous constitutive autophagy has a crucial role in maintaining cardiac structure and function during aging by controlling the quality of proteins and mitochondria. Cardiac-specific autophagy-related 5–deficient mice begin to die after the age of 6 months and exhibit a significant increase in the left ventricular dimensions. Moreover, these mice also have a decrease in the fractional shortening of the left ventricle at the
age of 10 months compared with control mice. The cardiac-specific autophagy-related 5–deficient mice also show a disorganized sarcomere structure and collapsed mitochondria with decreased mitochondrial respiratory functions. In contrast, transgenic mice expressing autophagy-related 5 using CAG promoter have enhanced autophagy ubiquitously including in the hearts, exhibit an antiaging phenotype in whole body accompanied by reduced cardiac fibrosis and extend their lifespan. The treatment of rapamycin, a potent inducer of autophagy, enhances lifespan and preserves cardiac function with age in mice. These findings suggest that autophagy has an effective antiaging property by clearing cytoplasmic protein aggregates and dysfunctional organelles and is a therapeutic target for attenuating cardiac aging. In contrast, CMA activity is reduced during aging mainly because of decreases of lysosome-associated membrane protein 2A abundance, but the role of CMA in cardiac physiology and pathophysiology during aging remains to be explored.

Degradation of Nucleic Acids and Aging

Decreased macromolecular degradation is possibly involved in the increased cytokine production associated with aging. We reported that the impaired degradation of mt-DNA in the lysosomes leads to a TLR9-mediated inflammatory response in an autonomous manner after pressure overload, suggesting that impaired DNA degradation can provoke an inflammatory response in mice during aging-related pathogenesis. Thus, the impaired degradation of mt-DNA leads to an autonomous inflammation of cardiomyocytes. However, the functional roles of TLR9-dependent innate immunity in cardiac aging remain to be determined.

Although it remains unknown whether mRNA decay is involved in cardiac aging, RNA degradation of proinflammatory cytokines may regulate inflammation in aged cardiomyocytes. HuR and tristetraprolin stabilize and destabilize TNFα, respectively. Tristetraprolin-deficient mice develop generalized inflammation characterized by cachexia, spontaneous arthritis, dermatitis, and neutrophilia mainly caused by TNFα overexpression. We have reported that the signal transducers and activator of transcription 6–deficient mice exhibit left ventricular dilatation and cardiac dysfunction with increased apoptotic response caused by sustained TNFα induction via the inhibition of tristetraprolin induction 1 week after pressure overload.

Figure 5. Mechanistic scheme between macromolecular degradation and inflammation in cardiac aging. Macromolecular degradation induces or inhibits inflammation via multiple molecular mechanisms. Autophagy inhibits NOD-like receptor 3 (NLRP3) inflammasome activation. Ubiquitin–proteasome system (UPS) mediates cytokine production downstream membrane-bound toll-like receptors (TLRs) activated by pathogens, pathogen-associated molecular patterns (PAMPs), and DAMPs via nuclear factor (NF)-κB activation through inhibitor of NF-κB (IκB) degradation. Caspase-1 activation leads to inflammasome activation via processing of pro–interleukin (IL)-1β and pro–IL-1β. In contrast, mitochondrial-DNA (mt-DNA) degradation via mitophagy prevents TLR9 activation and cytokine production thereby causes anti-inflammatory effect. Finally, mRNA decay of tumor necrosis factor (TNF) α and IL-6 regulated by tristetraprolin (TPP) and Regnase-1 directly inhibits inflammation. These systemic inflammatory responses and angiotensin II (Ang II) synergistically induce cardiac aging that associates with alterations of macromolecular degradation inside vascular cells and cardiomyocytes. The functional roles of macromolecular alterations in cardiomyocytes are largely unknown and should be determined in future. ASC indicates apoptosis-associated speck-like protein containing a caspase recruitment domain; MyD88, myeloid differentiation primary response 88; STING, stimulator of interferon genes; and TRAF6, TNF receptor-associated factor 6.
In contrast, miRNA may be involved in cardiac aging. As mentioned above, miR-34a promotes age-related cardiomyocyte cell death and cardiac dysfunction. miR-17-3p diminishes cardiac aging in mice. miR-22 and miR-18/19 may involve cardiac fibroblast senescence, positively and negatively, respectively.

Perspectives and Concluding Remarks

The increasing incidence of age-related disease that is inevitable for increased human longevity represents major social and medical challenges. Various mitochondrial defects such as mt-DNA mutations are found to accompany aging. Although life span is shortened in the homozygous mt-DNA mutant mice, the mt-DNA mutation load is much higher than that detected in elderly humans. Whether mitochondrial defects cause aging remains unclear. Recently, a group demonstrated that the level of growth differentiation factor 11 declines with age and that growth differentiation factor 11 plays a role in reversal of age-related cardiac hypertrophy. However, the other group was unable to confirm the findings. Growth differentiation factor 11 also inhibits skeletal muscle regeneration. These findings suggest that the role of growth differentiation factor 11 in aging is controversial. Here, we have described the current understanding of protein and nucleic acid degradation systems in maintaining cellular homeostasis and its role in cardiac aging (Figure 5). Cardiac aging is an intricate process accompanied by the cellular senescence of multiple cell types, each exhibiting a distinct pattern. Notably, cellular senescence on cardiomyocytes remains virtually unknown. There are multiple unresolved questions remaining, such as the following: (1) What is an appropriate marker for cardiomyocyte senescence? (2) How do the degradation systems decline with advancing age? (3) Is sterile inflammation in cardiomyocytes a target for cardiac aging? and (4) Does the maintenance of macromolecular degradation prevent cardiac aging?

A large body of data suggests that the immune response is highly dysregulated in elderly individuals. The dysfunctional degradation of macromolecules oxidized by increased ROS could lead to inflammasome activation or TLR stimulation to induce SASP, a form of sterile inflammation. Sterile inflammation has been reported to be regulated in the genesis of cardiac dysfunction and several other age-related diseases, such as atherosclerosis, diabetes mellitus, autoimmune diseases, neurodegenerative diseases, and cancer. Because serum proinflammatory cytokines are increased in the elderly, it is important to reveal the molecular mechanism to regulate the sterile inflammation associated with aging. Increased understanding through addressing the unanswered questions outlined above will uncover novel therapeutic approaches that can be used to prevent or slow cardiac aging progression.

Sources of Funding

This work was supported by grants CH/11/3/29051 and RG/11/12/29052 from the British Heart Foundation to Kinya Otsu.

Disclosures

None.

References


Macromolecular Degradation Systems and Cardiovascular Aging

Hiroyuki Nakayama, Kazuhiko Nishida and Kinya Otsu

Circ Res. 2016;118:1577-1592
doi: 10.1161/CIRCRESAHA.115.307495

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/118/10/1577

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/