Molecular mechanisms of cardiac aging

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Abstract
Age-associated changes in cardiovascular structure/function are implicated in the markedly increased risk for cardiovascular disease in older persons. Aging not only prolongs exposure to several other cardiovascular risks, but also leads to intrinsic cardiac changes, which reduces cardiac functional reserve, predisposes the heart to stress and contributes to increased cardiovascular mortality in the elderly. Intrinsic cardiac aging in the murine model closely recapitulates age-related cardiac changes in humans, including left ventricular hypertrophy, fibrosis and diastolic dysfunction. Cardiac aging in mice is accompanied by accumulation of mitochondrial protein oxidation, increased mitochondrial DNA mutations, increased mitochondrial biogenesis, as well as decreased cardiac SERCA2 protein. All of these age-related changes are significantly attenuated in mice overexpressing catalase targeted to mitochondria (mCAT). These findings demonstrate the critical role of mitochondrial reactive oxygen species (ROS) in cardiac aging and support the potential application of mitochondrial antioxidants to cardiac aging and age-related cardiovascular diseases.

Introduction
Cardiovascular diseases are the leading causes of death, and the elderly (>65 y) account for greater than 80% of patients with ischemic heart disease, more than 75% of patients with congestive heart failure, and greater than 70% of patients with atrial fibrillation.1 The exponential increase in mortality rate related to cardiovascular diseases in the geriatric population implies that cardiac aging per se is a major risk factor for cardiovascular diseases. Intrinsic cardiac aging is defined as the slowly progressive age-dependent degeneration and decline in function which makes the heart more vulnerable to stress and contributes to increased cardiovascular mortality and morbidity in the elderly. However, intrinsic cardiac aging can be obscured by the cardiomyopathic changes seen in diabetes or hypertension, which are highly prevalent in the elderly population. Both diabetes and hypertension have been shown to accelerate cardiovascular senescence.2,3 However, intrinsic cardiac aging is also evident in rodents, even though diabetes, hypertension or elevated blood cholesterol is absent in many species, including mouse. Furthermore, the availability of genetically modified mice and the relatively short mouse lifespan have made mouse a premier model of mammalian aging for gerontologic studies, including those of intrinsic cardiac aging.

Pathophysiology of cardiac aging
Data from the Framingham Heart Study and the Baltimore Longitudinal Study on Aging (BLSA) demonstrate that the prevalence of left ventricular hypertrophy increases with age. Diastolic function, measured by the ratio of early to late ventricular filling (E/A) by Doppler echocardiography also declines with age. While systolic function (ejection fraction) is relatively preserved in subjects at rest, the maximal exercise capacity decreases with age and left ventricular (LV) wall thickness increases progressively with age in both sexes, indicating increasing LV hypertrophy.4,5 Since the BLSA focused on subjects without hypertension or clinically apparent cardiovascular diseases, all of the above changes are likely manifestations of intrinsic cardiac aging. LV early diastolic filling (peak tissue E wave, Ea) is progressively compromised in age, which might be due to fibrosis and reduced ventricular compliance, coupled with slower reuptake of cytosolic calcium in myocardial cells, which further delays relaxation. The decline in early diastolic filling necessitates that atrial contraction during late diastolic phase (A wave; measured by tissue Doppler imaging as Aa) contribute to a larger fraction of LV filling. This likely increase atrial pressure and contribute to atrial hypertrophy, which can subsequently predispose to atrial fibrillation, the prevalence of which also increases with age. The decline in early diastolic filling is interpreted clinically as an evidence of diastolic dysfunction, defined as Ea/Aa <1.6 Diastolic dysfunction contributes to exercise intolerance in the elderly population and also predisposes to the development of diastolic heart failure. Diastolic heart failure, defined as symptoms of heart failure in the setting of preserved systolic function but impaired diastolic function, is prevalent in older individuals and markedly increases the risk of mortality.6-8
has been shown that diastolic heart failure accounts for more than 50% of patients over the age of 75 with the clinical diagnosis of congestive heart failure. In many individuals this was clinically unrecognized and untreated. Cardiac aging in the murine model closely recapitulates the age-related cardiac changes in apparently healthy human population noted above. Echocardiography performed on a mouse longevity cohort demonstrated that there were significant age-dependent linear trends in increased left ventricular mass index (LVMI) and left atrial dimension, reduction in fractional shortening (FS) and diastolic function (Ea/Aa), as well as worsening of myocardial performance index (MPI). LVMI increased by around 75% in the oldest group compared to a young adult group, indicating the increase prevalence of left ventricular hypertrophy with age. Systolic function measured by FS showed a modest decline of ~10% from the young adult to the oldest group. There is a substantial and significant decline of Ea/Aa with age, and the prevalence of diastolic dysfunction was dramatically increased to 55% in the oldest age group. As a consequence of the age-dependent decline of systolic and diastolic function, which increases left ventricular end-diastolic pressure, there is an enlargement of left atrium with age. The myocardial performance index was significantly increased (worsened) with age, indicating that a greater fraction of systole is spent to cope with the pressure changes during isovolemic phases. This has been shown to reflect both LV systolic and diastolic dysfunction. In summary, echocardiographic findings indicate that the murine heart becomes hypertrophic with age and the decline in diastolic function is prominent. While systolic function only declines slightly with age, the maximal exercise capacity and O2 consumption also significantly decline with age. Furthermore, there is a significant decline in general myocardial performance, shown by worsening (increase) in myocardial performance index (MPI).

Histopathologic changes in mouse hearts with age include subendocardial and interstitial fibrosis, hyaline cytoplasmic change, vacuolization of cytoplasm, variable and hypertrophic myocyte fiber size, collapse of sarcomeres, mineralization, and arteriosclerosis. These can be designated as age-associated cardiomyopathy. Morphometric analysis demonstrates cardiomyocytes hypertrophy (increased myocardial fiber size), increased cardiomyocytes apoptosis and increased fibrosis and amyloid deposition with age. Interestingly, fibrosis in old mouse hearts was more commonly observed in the ventricular subendocardium, which might be due to exposure to higher wall stress in the endocardial layers.

Molecular mechanisms of cardiac aging

Renin angiotensin aldosterone system (RAAS)

RAAS activation has been implicated in a broad spectrum of cardiovascular diseases, including hypertension, coronary heart disease and congestive heart failure, as well as atrial fibrillation. The prevalence of all of the above diseases has been shown to increase with age in the Framingham Heart Study. Indeed, Angiotensin II (Ang II) directly induces cardiomyocyte hypertrophy and apoptosis, increases cardiac fibrosis and impairs cardiomyocyte relaxation, consistent with the changes found in cardiac aging. Cardiac Ang II concentrations increase significantly in aged rodent hearts, probably related to increased tissue levels of angiotensin II converting enzyme (ACE). Though the mechanism of increased ACE in the aged heart is not well understood, long-term inhibition with angiotensin receptor blockers or disruption of angiotensin receptor type I has been shown to reduce age-dependent cardiac pathology and prolong rat and mouse survival. Thus, the activation of RAAS might play a central role in cardiac aging and age-associated cardiovascular diseases.

Adrenergic signaling

Chronic activation of β-adrenergic signaling is well known to be deleterious to the heart. This activation enhances cardiac metabolic demand secondary to increase in heart rate, contractility, afterload (blood pressure) and wall stress. Several clinical trials have shown that inhibition of β-adrenergic signaling by β-blockers provides survival benefit in patients with heart failure. Stimulation of β-adrenergic receptors (G-protein coupled receptors) activates adenylate cyclase, a key enzyme producing c-AMP as a secondary messenger. There are several isoforms of adenylate cyclase and type 5 (AC5) is the major form in the heart. Mice with disruption of AC5 were shown to have prolonged lifespan, likely mediated through upregulation of the Raf-1/pMEK/pERK pathway, which confers protection against stress, including oxidative stress. These mice were also shown to be protected from cardiac aging, including age-dependent cardiac hypertrophy, systolic dysfunction, apoptosis and fibrosis. Consistent with this, AC-5 disruption protected against chronic pressure overload-induced cardiac hypertrophy, apoptosis and failure by chronic catecholamine stimulation or aortic banding.

Insulin/IGF1 signaling

Insulin/IGF-1 signaling has been implicated in the regulation of lifespan in vertebrate and invertebrate animal models. Both mice deficient in growth hormone and mice with mutation of the IGF-1 receptor have been shown to have prolonged lifespans. Furthermore, deficiency in IGF-1 signaling was shown to improve cardiac performance at advanced age in Drosophila as well as to attenuate age-associated cardiomyocyte dysfunction in mice. This contrasts with findings from the Framingham Heart Study, which showed that low serum IGF-1 levels were associated with increased risk of heart failure in elderly subjects with-
out a history of myocardial infarction. Moreover, low levels of serum GH and IGF-1 have been correlated with systolic dysfunction in heart failure patients. Consistent with this, GH replacement therapy improves heart failure symptoms and attenuates cardiac remodeling in human patients and in the rat model of experimental heart failure. It also attenuates age-associated diastolic dysfunction and increases cardiac angiotensin II in senescent rats. Thus, further studies are required to address the controversial role of insulin/IGF1 signaling on cardiac aging.

Mitochondrial ROS in cardiac aging

Harman et al first proposed the free radical theory of aging more than five decades ago, postulating that the production of reactive oxygen species (ROS) is a major determinant of lifespan. The deleterious effects of ROS on various cell and organ components might drive an age-dependent functional decline of cells and organ systems, leading to associated degenerative diseases. Within cells, ROS are produced in multiple compartments and by multiple enzymes including NADPH oxidase at the plasma membrane, oxidative phosphorylation within mitochondria, and by cyclooxygenases and xanthine oxidase in the cytoplasm. Although all of these sources contribute to the overall oxidative burden, mitochondria contribute the majority of ROS generation as a byproduct of electron transfer during oxidative phosphorylation. Most specifically, excess electrons from complex I and III can be transferred directly to oxygen to generate superoxide anion (O$_2^-$), which is then converted to H$_2$O$_2$ by mitochondrial manganese superoxide dismutase. Mitochondrial H$_2$O$_2$ can diffuse into cytosol and nucleus, to activate redox-sensitive signaling. However, H$_2$O$_2$ is reduced by the Fenton reaction (Fe$^{2+}$ chemistry) into a hydroxyl radical (OH$^-$), the most reactive ROS species. Mitochondrial nucleic acids, lipids and proteins can be at highest risk from such damage. This has led to the mitochondrial variant of the free radical theory of aging, which proposes that mitochondrial ROS attack mitochondrial constituents, causing mitochondrial DNA damage and mitochondrial dysfunction, followed by a vicious cycle between increased mitochondrial damage and further production of ROS, which cause functional declines of cells and organ systems that eventually lead to death. Several studies have documented age-dependent impairment of mitochondrial function, mainly the decline in mitochondrial respiratory capacity (state 3) due to diminished activity of complexes I and IV, but relatively unaffected complexes II, III and V. This slower rate of mitochondrial electron transfer with age also favors mitochondrial superoxide production, leading to a positive feedback between complex I inhibition, mitochondrial ROS production (designated as ROS induced ROS release), mitochondrial DNA mutation and protein damages (reviewed by Navarro and Boveris).

Because the heart is a vital organ with high metabolic demand and rich in mitochondria, it is especially vulnerable to mitochondrial oxidative damage. Indeed, mitochondrial energetics deficiency has been widely documented in heart failure in both human patients and mouse models. The mechanisms may include mitochondrial biogenesis that is inadequate to match the increasing demand/workload, increased mitochondrial uncoupling and decreased substrate availability, and increased mitochondrial DNA deletions. In aging, mitochondrial DNA point mutation and deletion frequencies increase ~3 fold in the old mouse hearts, compared with young adult hearts. In addition, protein carbonyls in mitochondrial extracts, indicative of mitochondrial protein oxidative damage, significantly increase in the aged-heart. Oxidative damage to mitochondria is reflected in abnormal ultrastructure of mitochondria in old wild-type (WT) hearts, which showed disrupted cristae and vacuolation (loss of electron density). This damage stimulates signaling for mitochondrial biogenesis, seen in the aged heart by an increase in mtDNA copy number concomitant with significant upregulation of the master regulator PPAR- Coactivator-1-a (PGC-1a) and its downstream transcription factors mitochondrial transcription factor A (TFAM) and nuclear respiratory factors (NRFs). Direct evidence for the critical role of mitochondrial ROS in cardiac aging was reinforced by our experiments using mice overexpressing catalase targeted to the mitochondria (mCAT). mCAT mice had an 18% prolongation of lifespan, while mice overexpressing wild-type peroxisomal catalase (pCAT) did not and were significantly protected from the age-dependent increase in LVMI, decline in systolic and diastolic function, and increase in the prevalence of diastolic dysfunction, enlargement of left atrium, as well as worsening of myocardial performance. mCAT also attenuates age-dependent mitochondrial oxidative damage, as displayed by significant reductions of mtDNA mutation and deletion frequencies, decreased mitochondrial protein carbonyls, better protection of ultrastructure of mitochondrial cristae and attenuation of age-dependent activation of mitochondrial biogenesis.

Another line of evidence indicating the role of mitochondria in aging was demonstrated by mice with homozygous mutation of mitochondrial polymerase gamma, which impairs the proofreading capacity of the enzyme and thus induces a substantial increase in mtDNA point mutations and deletions. These mice had shortened lifespan and a phenotype of accelerated aging, including kyphosis, alopecia, anemia, osteoporosis and age-dependent cardiomyopathy. The accumulation of mitochondrial DNA mutations have been shown to increase apoptotic rate, and observations that mitochondrial damage and cardiomyopathy in these mice can be partially rescued by mCAT suggests that it is at least partially mediated through mt-ROS. Furthermore, it has been shown that accumulation of mtDNA deletions is better correlated with the premature aging phenotype in these mice than are mtDNA point mutations.
human, age-associated accumulation of mtDNA deletions have been documented in various tissues, including heart.\textsuperscript{36,37}

**Mechanism of age-dependent LV hypertrophy and diastolic dysfunction**

Cardiac hypertrophy involves a complex network of molecular signaling.\textsuperscript{38} Calcineurin is a phosphatase that dephosphorylates and activates the transcription factor NFAT, which then translocates into nucleus and interacts with several other transcription factors (e.g., GATA4) to initiate transcription of hypertrophic genes, such as atrial natriuretic peptides and brain natriuretic peptides.\textsuperscript{39}

Calcium handling proteins regulate the electro-mechanical coupling of cardiomyocytes. In old rodent hearts, we and others demonstrated the decline of sarcoplasmic reticulum ATPase (SERCA2) protein concentration (Xu and Narayanan 1998), concomitant with compensatory increase in the levels of Na+/Ca\textsuperscript{2+} exchanger.\textsuperscript{40} Oxidative damage to particular cysteine thiols could also impair SERCA2 activity.\textsuperscript{40} Chronic reduction of SERCA protein level/function could lead to prolongation of Ca\textsuperscript{2+} decay rate, reduction in SR Ca\textsuperscript{2+}-load and hence smaller amplitude of Ca\textsuperscript{2+} transients.\textsuperscript{41} Ca\textsuperscript{2+} transient changes and that decreased SERCA2 protein concentration is a predominant factor associated with age-dependent diastolic dysfunction and the aged heart might utilize the compensatory increase in the L-type Ca\textsuperscript{2+} currents\textsuperscript{42} and the significant prolongation of action potential duration to preserve SR loading and to keep the amplitude of intracellular Ca\textsuperscript{2+}-transients and contractions in old cardiomyocytes.\textsuperscript{42} The other factors contributing to age-dependent diastolic dysfunction include increased myocardial stiffness related to cardiac hypertrophy and fibrosis.

**Increased susceptibility to stress in the aged heart**

The aged myocardium is more susceptible to ischemia and hemodynamic stress than young myocardium.\textsuperscript{43} Cells from aged hearts have a lower threshold for ROS induced ROS release and increased susceptibility to mitochondrial permeability transition pore (MPTP) induction.\textsuperscript{44} MPTP is a voltage-dependent, high conductance "channel" located in the inner mitochondrial membrane. In the fully open state, it allows passive diffusion of solutes with molecular masses up to 1.5 kDa. MPTP opening may cause mitochondrial swelling, collapse of mitochondrial membrane potential (ΔΨm), ATP depletion, and eventually trigger apoptosis and/or cell death.\textsuperscript{45} Furthermore, ischemic preconditioning, the endogenous cardioprotective mechanism incited by repetitive ischemia that reduced the area of myocardial infarction, is impaired in the aged myocardium.\textsuperscript{46} The mechanism underlying this impairment might include decreased mitochondrial heat shock protein-70,\textsuperscript{46} reduced bioavailability of nitric oxide,\textsuperscript{47} damaged mitochondria that are vulnerable to stress, and diminished PKC translocation, all of which are believed to be required for the protective effect of ischemic preconditioning.\textsuperscript{48,49}

**Reduced regenerative capacity of the aged heart**

Several studies have demonstrated that resident stem and progenitor cells in the adult heart are capable of regeneration.\textsuperscript{50,51} Transplantation of these cells has been shown to improve cardiac function in rodent models of experimental myocardial infarction.\textsuperscript{52} Although these cells are capable of continuously repopulating cardiomyocytes in adult hearts, they fail to prevent the progression of cardiovascular diseases. One possible explanation is the aging of cardiac stem cells, which might reduce their number and impair their regenerative capacity, either by senescence of the stem cell or as a consequence of a hostile niche in the aged heart. Cardiac stem cells in older animals and patients with cardiovascular diseases had a higher rate of apoptosis, shorter telomeres and increased expression of the senescence marker p16INK4a. Furthermore, a recent study measuring 14C labeling (a retrospective birth dating method) in human hearts showed that the turnover or renewal rate of cardiomyocytes in young adults was approximately 1% annually, and this was significantly reduced to 0.45 % in the hearts of the elderly.\textsuperscript{53} Thus, the decline in number and regenerative capacity of cardiac stem cells might explain part of the increased susceptibility of the elderly to heart failure.

**Cardiac aging in other model organisms**

The emerging new techniques for analysis of Drosophila cardiac function in recent years has allowed the fruit fly genetic system to be used to study age-related functional changes in cardiac tissue. The Drosophila heart demonstrates an age-dependent decline in heart rate and contractility, increase in susceptibility to arrhythmia and pacing induced cardiac failure. Although the mouse has been a premier model for mammalian aging studies because of the availability of genetically modified mice and the relatively short mouse lifespan, research using nonhuman primates provides a valuable tool to investigate aging process which closely recapitulate human aging and allows the investigation of potential anti-aging interventions before human clinical trials. Longitudinal study of aging in rhesus monkeys (Macaca mulatta) conducted by the National Institute of Aging revealed that under normal diets rhesus monkeys develop several aging-related pathologies, including aortic and mitral valves degenerative calcifications, loss or degeneration of myocardial fibers with hypertrophy of remaining cardiomyocytes, lipofuscin accumulation and variable degrees of myocarditis, multifocal interstitial fibrosis, myocardial infarction and congestive heart failure.\textsuperscript{54-56}

**References**


