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# The Aging Heart

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Aging results in progressive deteriorations in the structure and function of the heart and is a dominant risk factor for cardiovascular diseases, the leading cause of death in Western populations. Although the phenotypes of cardiac aging have been well characterized, the molecular mechanisms of cardiac aging are just beginning to be revealed. With the continuously growing elderly population, there is a great need for interventions in cardiac aging. This article will provide an overview of the phenotypic changes of cardiac aging, the molecular mechanisms underlying these changes, and will present some of the recent advances in the development of interventions to delay or reverse cardiac aging.

Cardiovascular diseases are the leading cause of death in most developed nations. Although it has received the least public attention, aging is by far the dominant risk factor for development cardiovascular diseases, as the prevalence of cardiovascular diseases increases dramatically with increasing age. The prevalence rate of cardiovascular diseases is >70% for Americans 60 to 79 years of age and >80% for Americans >80 years of age (Go et al. 2014). Even without associated systemic risk factors, intrinsic cardiac aging leads to structural and functional deteriorations of the heart in elderly individuals. Therefore, interventions to combat cardiac aging will not only improve healthspan of the elderly but can also extend lifespan by delaying cardiovascular disease-related deaths. Although there is presently no treatment for cardiac aging, recent advances in the understanding of the mechanisms of cardiac aging have provided new insights, and we are now poised on the threshold of development of new interventions to attenuate or reverse cardiac aging.

## CARDIAC AGING IN HUMAN AND ANIMAL MODELS

The Framingham Heart Study and the Baltimore Longitudinal Study on Aging (BLSA) have shown that, in healthy individuals without concomitant cardiovascular diseases, aging results in an increase in the prevalence of left ventricular (LV) hypertrophy, a decline in diastolic function, and relatively preserved systolic function at rest but a decline in exercise capacity, as well as an increase in the prevalence of atrial fibrillation (Lakatta and Levy 2003b). These changes can be independent of conventional risk factors for heart disease (smoking, hypertension, blood lipid levels, diabetes, etc.) and, thus, may be considered to be part of intrinsic cardiac aging. At rest, systolic function measured by the ejection fraction (EF) re-

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mains steady in older populations. However, on exercise, maximum heart rate and EF are lower in older populations, indicating reduced cardiac reserve (Lakatta 2002). An age-dependent increase in myocardial performance index (MPI) has also been shown (Spencer et al. 2004). An increase in MPI indicates that a greater fraction of systole is spent to cope with the pressure changes during isovolumetric phases, and has been shown to reflect both LV systolic and/or diastolic dysfunction (Tei et al. 1997). Because of impaired early diastolic filling and an increased contribution of atrial contraction to LV filling, the peak early filling velocity and the ratio of the early and late (E/A ratio) filling velocity decrease with age; the early component is larger than the late atrial component of filling in young persons, but, when this reverses, it is an indicator of diastolic dysfunction (Downes et al. 1989; Swinne et al. 1992; Kitzman 2002; Choi et al. 2009). Diastolic dysfunction is increasingly seen in the elderly in the absence of systolic heart failure, a condition that has been given the designation of heart failure with preserved ejection fraction (HFpEF). It is especially prevalent in aged women (Brouwers et al. 2012) and is an increasing cause of hospital admissions (Oktay et al. 2013).

Rodents, particularly the mouse model, are widely used in cardiac aging studies. Murine cardiac aging phenotypes closely recapitulate the phenotypes of human cardiac aging (Lakatta and Levy 2003a). Echocardiography performed on a mouse longevity cohort showed that left ventricular mass index (LVMI) and left atrial dimension significantly increased with age. Diastolic function measured by tissue Doppler declines with age, whereas systolic function showed a modest reduction from young adult to the oldest group. The MPI also worsens with age, which is consistent with the age-related declines in systolic and diastolic function (Barger et al. 2008). In addition to the similar cardiac aging phenotypes, the relatively short lifespan and the availability of genetically modified mice are the advantages of using mouse model in the study of the molecular mechanisms of cardiac aging. Despites sharing similar cardiac aging phenotypes as human, laboratory mice do not develop elevated blood pressure or adverse blood glucose and lipid profiles (Zheng et al. 2003; Dai et al. 2009). This allows the intrinsic cardiac changes of aging to be investigated without the added complications of cardiovascular risk factors, including hypertension and diabetes.

## MOLECULAR MECHANISMS OF CARDIAC AGING

Recent studies have shown the involvements of multiple molecular mechanisms in the pathogenesis of cardiac aging. These mechanisms are summarized in Figure 1 and discussed below.

### Altered Nutrient and Growth Signaling

Cardiac hypertrophy is a hallmark of cardiac aging. Deregulation of nutrient and growth signaling pathways, including mechanistic target of rapamycin (mTOR) and insulin-like growth factor-1 (IGF-1) signaling, have been implicated in cardiac hypertrophy and aging. mTOR integrates nutrient and hormonal signals to regulate growth and is a major modulator of aging and age-related disease (Kennedy et al. 2007). Previous studies in *Drosophila* and mouse models have shown that increased mTOR signaling impairs and reduced mTOR signaling improves resistance to cardiac aging. Bodmer's laboratory initially showed that inhibition of the mTOR pathway could attenuate the age-related decline in cardiac function in *Drosophila* (Luong et al. 2006). They later showed that eukaryotic translation-initiation factor 4E (eIF4E)-binding protein (4EBP) overexpression attenuates the age-related decline to a similar extent as overexpression of the TOR antagonist tuberous sclerosis complex (TSC), and overexpression of eIF4E leads to an accelerated decline of myocardial function (Wessells et al. 2009). These findings implicate a major role of mTOR/eIF4E signaling in cardiac aging in *Drosophila*. In addition, Meikle et al. (2005) showed that mice with cardiac-specific deletion of TSC1, a model of increased mTOR signaling, develop dilated cardiomyopathy and have a median lifespan of 6 mo. Although there is no evidence yet on the

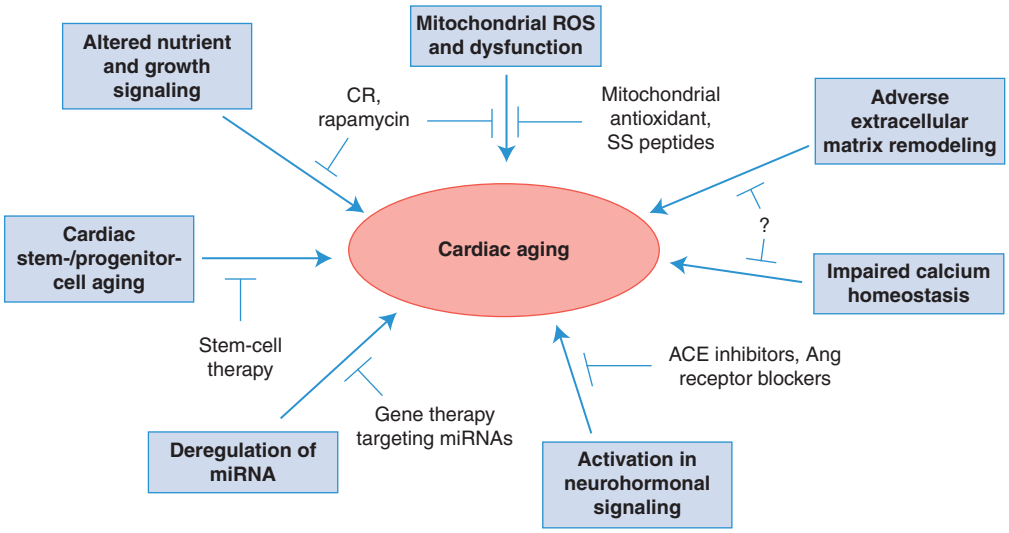


**Figure 1.** A schematic summary of the molecular mechanisms of cardiac aging and potential cardiac aging interventions. ROS, Reactive oxygen species; CR, calorie restriction; SS, Szeto–Schiller; miRNA, microRNA; ACE, angiotensin-converting enzyme; Ang, angiotensin.



beneficial effects of genetic manipulation to decrease mTOR activity in the aging mammalian heart, inhibition of mTOR signaling by caloric restriction (CR) or rapamycin (see below) has been shown to protect against cardiac aging.

Insulin/IGF-1 signaling is one of the best-characterized pathways of lifespan regulation in animal models. Deficiency in insulin/IGF-1 signaling improves cardiac performance at advanced age in *Drosophila* and attenuates age-related cardiomyocyte dysfunction in mice (Wessells et al. 2004; Li et al. 2008). However, in humans, an age-dependent decline in serum IGF-1 levels (Corpas et al. 1993) correlates with an increased risk of heart failure among elderly patients without prior history of heart disease (Vasan et al. 2003), and interventions that increase IGF-1 signaling, such as growth hormone therapy, may be beneficial in heart failure (Broglio et al. 1999; Khan et al. 2002). The beneficial effects of IGF-1 on cardiovascular disease may be conferred by mitochondrial protection mechanisms. One study showed that in vitro treatment of endothelial cells and cardiomyocytes with IGF-1 decreased mitochondrial superoxide production (Csiszar et al. 2008). Furthermore, low-plasma levels of growth hormone (GH) and IGF-1 in Ames dwarf mice are associated with increased mitochondrial oxidative stress in the vasculature and the heart, which is responsible for the impaired contractile function (Ren and Brown-Borg 2002). Recent studies show that IGF-1 treatment of aged rats protects against mitochondrial oxidative stress (Puche et al. 2008), and other studies suggest that interventions that increase circulating IGF-1 levels exert cardiovascular protective effects in aging (Rivera et al. 2005; Groban et al. 2006; Lopez-Lopez et al. 2007). The roles of mitochondrial oxidative stress in cardiac aging are discussed further below.

## Mitochondrial Oxidative Damage and Mitochondrial Dysfunction

The mitochondrial free-radical theory of aging proposes that excessive mitochondrial reactive oxygen species (ROS) damages mitochondrial DNA and redox-sensitive mitochondrial proteins, causing mitochondrial dysfunction and further increase in ROS production (the "vicious cycle" of ROS-induced ROS release), and that this oxidative damage leads to cellular

and organ functional declines that limit lifespan and healthspan (Harman 1972).

Cardiomyocytes, being postmitotic, are highly susceptible to age-related mitochondrial damage. Mitochondria in aged cardiomyocytes are usually enlarged with swelling, loss of cristae, and even destruction of inner membranes and are deficient in ATP production (Terman and Brunk 2004). A previous study has shown that mitochondrial production of ROS significantly increases in the heart with advanced age (Judge et al. 2005). Also, increasing evidence suggests that abnormal mitochondrial ROS production and impaired ROS detoxification contribute to mitochondrial dysfunction and cardiomyopathy in old age (reviewed in Terzioglu and Larsson 2007; Trifunovic and Larsson 2008; Mammucari and Rizzuto 2010).

Direct evidence supporting the role of mitochondrial oxidative damage in cardiac aging was provided by mice overexpressing catalase targeted to the mitochondria (mCAT) (Schriner et al. 2005; Dai et al. 2009). In addition to an extension of median and maximum lifespan, mCAT mice displayed greatly attenuated cardiac aging phenotypes, including reduced cardiac hypertrophy and improved diastolic function and myocardial performance (Dai et al. 2009). The attenuated cardiac aging phenotypes in mCAT mice were accompanied by significantly reduced mitochondrial protein oxidative damage and mitochondrial DNA mutation and deletion frequencies, suggesting prevention of mitochondrial oxidative damage as a strategy for protection from cardiac aging. Additional evidence is provided by mice with homozygous mutation of mitochondrial polymerase γ ($Polg^{m/m}$), which have substantial increases in mtDNA mutations and deletions with age (Trifunovic et al. 2004; Kujoth et al. 2005). $Polg^{m/m}$ mice have a shortened lifespan and develop cardiomyopathy in middle age (13–14 mo) (Trifunovic et al. 2004; Dai et al. 2010). Interestingly, mCAT partially rescues the mitochondrial damage and cardiomyopathy in $Polg^{m/m}$ mice, further supporting the role of mitochondrial ROS in cardiac aging (Dai et al. 2010).

Peroxisome proliferator-activated receptor γ coactivator 1α (PCG-1α) is the key regulator of mitochondrial biogenesis, and PCG-1α enhances mitochondrial function in the heart (Wenz 2011). PCG-1α expression is repressed in the failing heart, and PCG-1α knockout mice have reduced mitochondrial gene expression and develop cardiac dysfunction at 7 mo of age (Arany et al. 2005). Cardiac-specific overexpression of PCG-1α in adult mice nevertheless leads to cardiomyopathy (Russell et al. 2004).

Given the complexity of the systems involved, mitochondrial dysfunction and aberrant ROS production likely contribute to aging through both direct damage to cellular macromolecules and interference with normal signaling and energetics. The effect of mitochondrial ROS in signaling and energetics in cardiac aging was previously reviewed (Dai et al. 2014a).

## Adverse Extracellular Matrix (ECM) Remodeling

ECM is a complex collection of proteins located outside the cells and provides structural and biological supports to the surrounding cells. Cardiac fibroblasts are the primary sources of cardiac ECM proteins, including collagen types I, II, III, IV, V, and VI, elastin, fibronectin, laminin, and fibrinogen (DeQuach et al. 2010). Cardiac ECM aligns cardiomyocytes and provides structural support to the heart; however, excessive ECM deposition increases the stiffness of the myocardium and mediates diastolic dysfunction (Ouzounian et al. 2008). ECM composition is dynamically remodeled by the balance of the synthesis and degradation of ECM proteins by matrix metalloproteinases (MMPs) and other proteases. Cardiac aging is associated with myocardial fibrosis, and deregulation of ECM synthesis and degradation has both been observed in aging hearts.

Transforming growth factor-β (TGF-β) is a profibrotic factor that has been shown to induce the expression of ECM proteins and inhibit matrix degradation by MMPs (Bujak and Frangogiannis 2007). Reduced TGF-β1 expression results in reduced myocardial fibrosis and improved LV compliance in 24-mo-old TGF-β1 heterozygous mice (Brooks and Conrad 2000). Connective tissue growth factor (CTGF), an-

other profibrotic factor, is a downstream mediator from TGF-β and its expression increases with age (Wang et al. 2010). Mice overexpressing CTGF in a cardiomyocyte-specific manner show accelerated cardiac aging and begin to develop age-related cardiac dysfunction at 7 mo of age (Panek et al. 2009). The role of ECM synthesis in cardiac aging is also implicated by the accelerated myocardial fibrosis that accompanies higher TGF-β and CTGF levels in senescence-accelerated mice that display diastolic dysfunction at 6 mo of age (Reed et al. 2011). In another study, Bradshaw et al. (2010) showed that expression of a matricellular protein, secreted protein acidic and rich in cysteine (SPARC), increased with age, and that deletion of SPARC resulted in reduced fibrillar collagen content in the LV and decreased LV diastolic stiffness. Together, this evidence suggests that increased ECM synthesis is an important mediator of diastolic dysfunction with age and that reduced ECM synthesis can improve cardiac aging.

MMPs are a family of 25 zinc-dependent enzymes that regulate ECM degradation; tissue inhibitors of matrix metalloproteinase (TIMPs) are a family composed of TIMP-1, -2, -3, and -4, which regulate MMP proteolytic activity in the tissue (Tayebjee et al. 2005). The expression levels of MMPs and TIMPs are differentially regulated by age (Lindsey et al. 2005; Bonnema et al. 2007), but the roles of most MMPs and TIMPs in cardiac aging have not been established. Spinale and colleagues showed that cardiac-specific MT1-MMP overexpression accelerated cardiac aging responses in mice and that MT1-MMP-overexpressing mice have increased myocardial fibrosis and LV dysfunction at middle age (Spinale et al. 2009). More recently, Chiao et al. (2011) showed that MMP-9 levels increase in the LV and plasma of aged C57Bl6 mice, and that aged MMP-9 knockout mice display attenuated cardiac aging phenotypes, including reduced collagen deposition and preserved diastolic function (Chiao et al. 2012). The attenuated cardiac aging phenotypes in MMP-9 knockout mice are accompanied by reduced expression of profibrotic proteins, periostin, and CTGF and a compensatory increase in MMP-8 levels in the LV (Chiao et al. 2012). Together, these findings suggest a role of ECM degradation that is under complex regulation by MMPs in cardiac aging.

## Impaired Calcium Homeostasis

One mechanism underlying age-dependent diastolic dysfunction is impaired active relaxation of cardiomyocytes (Zile and Brutsaert 2002; Kass et al. 2004). During relaxation, calcium ions dissociate from the actin–myosin complex and are taken up into the sarcoplasmic reticulum (SR) or extruded outside the cardiomyocyte. Impaired $Ca^{2+}$ cycling, increased myofilament stiffness, reduced $Ca^{2+}$ sensitivity of myofilament proteins, and alterations in actin or myosin properties can lead to impaired cardiomyocyte relaxation (Zile and Brutsaert 2002; Kass et al. 2004; Borlaug and Kass 2006). Aged mouse hearts have reduced sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA2) expression (Dai et al. 2009) and activity (Janczewski and Lakatta 2010), with compensatory increase in the levels of the $Na^{+}/Ca^{2+}$ exchanger (Koban et al. 1998). Studies suggest that the aged heart uses the compensatory increase in the L-type $Ca^{2+}$ currents (Josephson et al. 2002) and prolongation of action potential duration to preserve SR loading and to maintain intracellular $Ca^{2+}$-transients and contractions in old cardiomyocytes (Janczewski et al. 2002). Posttranslational modifications of SERCA2, including age-related oxidation and nitration, have also been shown (Knyushko et al. 2005; Sharov et al. 2006), but their roles in cardiac aging are as yet unclear.

## Chronic Activation in Neurohormonal Signaling

The renin angiotensin aldosterone system (RAAS) is the key endocrine system regulating hypertension and stress-induced cardiac hypertrophy. Angiotensin II (Ang II) infusion induces cardiomyocyte hypertrophy, increases cardiac fibrosis, and impairs cardiomyocyte relaxation (Domenighetti et al. 2005); these responses closely recapitulate the age-related changes in the heart (Dai et al. 2009). Studies have showed that Ang II concentration significantly

increased in the aged rodent heart (Groban et al. 2006; Dai et al. 2009) potentially caused by increased tissue levels of angiotensin-converting enzyme (ACE) (Lakatta 2003). Moreover, long-term inhibition of Ang signaling by ACE inhibitors, angiotensin receptor blockers, or genetic disruption of Ang II receptor type I extend lifespan and delay age-dependent cardiac pathology in rodents (Basso et al. 2007; Benigni et al. 2009).

Activation of the β-adrenergic signaling increases heart rate, contractility, blood pressure, wall stress, and metabolic demand of the heart, and chronic stimulation of β-adrenergic signaling is deleterious to the heart. Deletion of adenylate cyclase type 5 (AC5), a key enzyme downstream from β-adrenergic signaling, extends murine lifespan and is protective against age-dependent cardiac hypertrophy, systolic dysfunction, apoptosis, and fibrosis (Yan et al. 2007).

### Other Potential Mechanisms

Increasing evidence suggests that microRNAs (miRNAs) are important regulators of aging and cardiovascular diseases (Smith-Vikos and Slack 2012; Quiat and Olson 2013), and several recent studies implicated the roles of miRNAs in cardiac aging. van Almen et al. (2011) showed that the expression of the miR-17-92 cluster (consisting of miR-18a, miR19a, and miR-19b) decreases, whereas the expression of their targets, CTGF and ECM protein thrombospondin-1 (TSP-1) increases, in heart-failure-prone C57Bl6 × 129Sv mice. In aged cardiomyocyte cultures, these investigators showed that miR-18a and miR-19b regulated expression of CTGF, TSP-1, and collagen, suggesting that these miRNAs mediate age-related ECM remodeling in the hearts. In C57Bl6 mice, Jazbutyte et al. (2013) detected an age-related increase in miR-22 in hearts and showed that miR-22 regulates cardiac fibroblast senescence. In a recent study, Boon et al. (2013) showed that expression of miR-34a increased in aged mouse hearts, and in vivo silencing of miR-34a for 1 wk rescued the increase in cardiomyocyte cell death in aged mice. They also showed that aged miR-34a knockout mice had improved contractile function and reduced cardiac hypertrophy compared to wild-type littermates. This evidence suggests that increased miR-34a expression in the aged heart contributes to cardiac aging.

Previous studies have shown that cardiac stem cells and progenitor cells may regenerate the adult heart to some extent (Beltrami et al. 2003; Hsieh et al. 2007). Cardiac stem cells in the aged heart may have impaired regenerative capacity, either by senescence intrinsic to the stem cells or by an extrinsic hostile microenvironment associated with advanced age (reviewed in Anversa et al. 2005; Ballard and Edelberg 2007). Torella and colleagues (2004) observed an increased proportion of senescent cardiac stem cells (which express senescent marker $p16^{ink4a}$ and have reduced telomere length) in old wild-type mice and showed that IGF-1 overexpression can prevent senescence of cardiac stem cells. A recent study also showed that attenuation of the IGF-1/IGF-1-receptor and hepatocyte growth factor/mesenchymal-epithelial transition factor (c-Met) systems mediates aging of cardiac progenitor cells (Gonzalez et al. 2008). In another study, Bergmann et al. (2009) measured $^{14}C$ from nuclear bomb tests in genomic DNA of human myocardial cells and used this method to show that the turnover or renewal rate of cardiomyocytes is reduced from 1% in young adult hearts to 0.45% in the hearts of the elderly. These results suggest that the regenerative capacity of cardiac stem cells declines with aging and that such declines may mediate the impaired myocardial repair in aged hearts.

## RECENT ADVANCES IN INTERVENTIONS FOR CARDIAC AGING

The improved understanding of the pathogenesis of cardiac aging may greatly advance the development of interventions that target specific mechanisms to delay or treat cardiac aging (Fig. 1).

### Calorie Restriction (CR)

CR is the most well-studied longevity intervention and has been shown to increase lifespan in a

wide array of model organisms, from yeast and nematodes to mice, rats, and (perhaps) rhesus monkeys (Colman et al. 2009; Cruzen and Colman 2009; Fowler et al. 2010; Kastman et al. 2010; McKiernan et al. 2011). CR is protective against a variety of age-related pathologies, including cardiovascular disease, in rodents and nonhuman primates (Cruzen and Colman 2009; Niemann et al. 2010; Shinmura et al. 2011a). An early study by Taffett et al. (1997) showed that CR had a large positive effect on age-related impairment of diastolic function in mice. Kemi et al. (2000) then found that moderate dietary restriction (35% reduction in calorie intake) can attenuate age-related cardiomyopathy in male Sprague–Dawley rats. A later study showed that human volunteers undertaking CR for a mean of 6.5 yr had reduced blood pressure and systemic oxidative stress, and improved diastolic function (Meyer et al. 2006). Similar improvements in diastolic function have been reproduced in individuals maintained on 1-yr CR (Riordan et al. 2008). In addition to the protective effects of long-term CR, our laboratory recently showed that CR for 10 wk was able to reverse the preexisting cardiac hypertrophy and diastolic dysfunction in old mice, and that this was accompanied by proteomic and metabolomic remodeling to a more youthful state (Dai et al. 2014b).

Multiple mechanisms have been implicated in the beneficial effects of CR including inhibition of mTOR signaling, normalization of mitochondrial biogenesis (Lopez-Lluch et al. 2006), attenuation of mitochondrial ROS production and the subsequent ROS-induced signaling (Nisoli et al. 2005; Ungvari et al. 2008; Csiszar et al. 2009; Shinmura et al. 2011b), and increased SIRT1 signaling (Lopez-Lluch et al. 2006, 2008). These have proven to be fertile areas for the study of pharmacologic interventions to enhance healthspan.

## Rapamycin

Although a large body of evidence supports the protective effects of CR in age-related pathologies including cardiac aging, the use of CR in humans would be challenging. Developing CR mimetics that mimic the beneficial effects of CR by targeting cellular metabolic and stress response pathways without actual restriction on calorie intake has been of great interest to the aging research community. mTOR plays a principle role in nutrient signaling and rapamycin is a well-established inhibitor of mTOR. The National Institute on Aging (NIA) Intervention Testing Program has recently shown that long-term rapamycin treatment initiated at 9 or 18 mo of age extended lifespan in mice with a mixed genetic background; these results were reproducible in three independent laboratories (Harrison et al. 2009; Miller et al. 2011). Subsequent studies have confirmed these results and have extended the studies to other measures of healthspan (Wilkinson et al. 2012).

With increasing evidence supporting the role of mTOR in aging and healthspan, the effects of rapamycin on cardiac aging are undoubtedly of interest to the aging research community. Rapamycin treatment for 1 yr initiated at mid-life attenuated the increased LV dimensions in aged hearts, but failed to show any effect on systolic function in male mice (Neff et al. 2013). Flynn et al. (2013) showed that rapamycin treatment for 12 wk initiated at late life can attenuate age-related cardiac hypertrophy and marginally improve systolic function in female mice, accompanied by a reduction in age-related inflammation. Recently, our laboratory showed that short-term rapamycin (10 wk) recapitulated the effect of CR and substantially improved both diastolic function and LV hypertrophy in old mice (Dai et al. 2014b). The reversal of cardiac aging phenotypes appeared to be mechanistically linked to proteomic and metabolic remodeling to increase mitochondrial protein content and reverse the age-related metabolic shift from fatty acid oxidation (FAO) to glycolysis and gluconeogenesis.

Further investigations on the mechanisms and kinetics of rapamycin benefits will be required to evaluate the potential of rapamycin as a pharmacological intervention to prevent, or even reverse, cardiac aging and its concomitant negative physiological consequences.

## Mitochondrial Intervention

Mitochondrial dysfunction and mitochondrial ROS are critical mechanisms in the age-dependent decline in cardiac function; therefore, interventions combating mitochondrial ROS and improving mitochondrial function are attractive targets for interventions in cardiac aging. The success of mCAT protection in cardiac aging, but not of peroxisomal catalase or the nontargeted antioxidant *N*-acetylcysteine, underscored the importance of mitochondrial specificity in antioxidant intervention (Dai et al. 2011). One approach for targeting antioxidants to mitochondria is to use the negative potential gradient across the inner mitochondrial membrane (IMM). The negative potential gradient across IMM allows lipophilic cations to penetrate the IMM and accumulate in the mitochondrial matrix. Triphenylalkylphosphonium ions ($TPP^+$) have been conjugated to coenzyme Q (MitoQ) and plastoquinone (SkQ1) (Skulachev et al. 2009; Smith et al. 2012) to deliver these redox-active compounds into the mitochondrial matrix. Although their effects on cardiac aging have not been established, MitoQ and SkQ1 have been shown to have beneficial effects in models of ischemia reperfusion and cardiac hypertrophy (Adlam et al. 2005; Bakeeva et al. 2008; Graham et al. 2009; Dikalova et al. 2010). A major limitation of these $TPP^+$-conjugated antioxidants is their dependence on mitochondrial potential, which is often compromised in pathological conditions. MitoQ and SkQ have also been shown to inhibit respiration and disrupt mitochondrial potential at concentrations above 25 μM, which limits their uptake (Kelso et al. 2001; Antonenko et al. 2008). Moreover, MitoQ is reduced to a semiquinone radical at complex I and can increase superoxide production (O'Malley et al. 2006; Murphy and Smith 2007; Scatena et al. 2007); this pro-oxidant activity of MitoQ must be carefully evaluated when considering this intervention.

Another approach for targeting an intervention to mitochondria can be performed by utilizing an affinity to a mitochondrial component. The Szeto–Schiller (SS) compounds are tetrapeptides with an alternating aromatic-cationic amino acids motif. Studies have shown that SS peptides preferentially concentrate in the IMM over 1000-fold compared with the cytosolic concentration (Zhao et al. 2004; Doughan and Dikalov 2007; Bakeeva et al. 2008). In contrast to MitoQ and SkQ1, the mitochondrial uptake of SS peptides is not dependent on mitochondrial potential, and they can concentrate even in depolarized mitochondria (Zhao et al. 2004; Doughan and Dikalov 2007). The most-studied SS peptide, SS-31 (D-Arg-2′, 6′-dimethyltyrosine-Lys-Phe-$NH_2$), was originally thought to exert its beneficial effect by the free radical scavenging activity of dimethyltyrosine (Graham et al. 2009). However, recent studies have revealed that SS-31 selectively binds to cardiolipin on the inner mitochondrial (Birk et al. 2013a,b; Szeto 2014). The binding of SS-31 to cardiolipin alters the interaction of cardiolipin with cytochrome *c*, and favors its electron carrier function while inhibiting its peroxidase activity (Birk et al. 2013a; Szeto 2014). SS-31 treatment increases ATP production, inhibits ROS generation, and prevents cardiolipin peroxidation and loss of cristae (Birk et al. 2013a). These findings suggest that the mitochondrial protective properties of SS-31 may be attributed to ROS-independent mechanisms, such as improved energetics, with reduction of ROS production as a secondary benefit. Our laboratory showed that the mitochondrial protective peptide SS-31 prevents pressure overload-induced cardiac hypertrophy as well as failure in a highly parallel manner to mCAT overexpression (Dai et al. 2011, 2012, 2013). Although the effects of SS-31 on cardiac aging have not been reported, recent studies from our laboratory have shown that 8-wk treatments of SS-31 can reverse age-related diastolic dysfunction in old mice (Chiao and PS Rabinovitch, unpubl.), supporting the therapeutic potential of SS-31 in cardiac aging.

## Inhibition of Renin Angiotensin Aldosterone Signaling

As noted above, Ang II concentrations increase in aged hearts, and Ang II infusion induces

structural, functional, and molecular changes similar to cardiac aging (Groban et al. 2006; Dai et al. 2009), highlighting the therapeutic potential of inhibition of Ang II signaling in cardiac aging. Basso et al. (2007) showed that long-term blockade of Ang II signaling by the angiotensin-converting enzyme inhibitor enalapril or by the angiotensin receptor type I inhibitor losartan can extend the lifespan of male Wistar rats and substantially attenuate age-related cardiovascular pathologies. In an earlier study, Inserra and colleagues (1995) showed that life-long treatment of enalapril can attenuate cardiac hypertrophy and interstitial fibrosis in hearts of 24-mo-old mice without significant changes in blood pressure. A later study by Stein et al. (2010) showed that long-term (10-mo) treatment with losartan, beginning at 12 mo of age, can also reduce myocardial fibrosis and fibrosis-related arrhythmias in aged mice. Groban et al. (2012) recently compared the effects of low-dose (non-blood-pressure lowering) enalapril and losartan for 6 mo initiated at 24 mo of age in male Fischer 344 × Brown Norway rats. They showed that, although low-dose enalapril and losartan both reduced cardiac oxidative stress, only enalapril was able to mitigate diastolic dysfunction, and they suggested that this may be mediated by a lowered ratio of phospholamban to SERCA2.

## GDF-11

Recently, Loffredo et al. (2013) showed, by heterochronic parabiosis, that the circulation of young mice can regress cardiac hypertrophy in aged mouse hearts. By proteomic analysis of plasma samples from young and old mice, they identified that growth differentiation factor 11 (GDF11) is a circulating factor that declines with age and may be responsible for the reversal of age-related hypertrophy in heterochronic parabiosis. Importantly, restoring circulating GDF11 levels of old mice to young levels, by daily intraperitoneal injection of recombinant GDF11 (rGDF11) for 30 days can also reverse age-related hypertrophy. Treatment with rGDF11 reduced hypertrophic markers (ANP and BNP) expression and increased SERCA-2 expression, recapitulating the molecular changes mediated by parabiosis (Loffredo et al. 2013). The precise mechanism of GDF11 action, its effect on age-related diastolic dysfunction, and the role of GDF11 in human cardiac aging remain to be investigated; however, the results from the mouse model suggest an exciting therapeutic potential of GDF11 in cardiac aging.

## Therapies Targeting miRNAs

As discussed above, recent studies suggest that miRNAs are important regulators of cardiac aging. With age, the expression of the miR-17-92 cluster (miR-18a, miR19a, and miR-19b) decreases, whereas the expressions miR-22 and miR-34a increase in hearts (Boon et al. 2013). Boon et al. (2013) showed that in vivo silencing of miR-34a by injection of antisense oligonucleotides (antagomirs) or locked nucleic acid (LNA)-based anti-miRs can reduce expression of miR-34a and partially rescue cardiac phenotypes in mice. This finding supports the potential of gene therapy to reverse the age-related changes in miRNAs to treat cardiac aging. However, as one miRNA is likely to have multiple targets, gene therapy targeting miRNA may trigger undesirable side effects. An alternative approach is to identify the miRNA targets that mediate cardiac aging responses and manipulate the specific target genes as treatment strategy.

## Cardiac Stem-Cell or Progenitor-Cell Therapy

The recent discovery that the heart is able to regenerate although cardiac stem cells and cardiac progenitor cells has attracted enormous attention to the potential of stem-cell therapy for cardiovascular diseases and aging. Two approaches for stem-cell therapy are (1) direct delivery of cardiac stem cells/cardiac progenitor cells (with or without treatment to enhance cardiac differentiation or regenerative capacity) to the heart, and (2) delivery of agents that enhance the function of endogenous cardiac stem cells or progenitor cells (Ballard and Edelberg 2007). Potential therapeutic agents for enhancing en-

dogenous stem-cell or progenitor-cell function include stromal-cell-derived factor (SDF)-1, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and IFG-1 (Ballard and Edelberg 2007). For direct stem-cell or progenitor-cell delivery, the therapeutic effects are limited by the proliferation, engraftment, survival, and persistence of the transplanted cells. In a recent study, Mohsin et al. (2012) used lentivirus transduction to overexpress Pim-1 kinase in cardiac progenitor cells isolated from a 68-yr-old heart failure patient, and showed enhanced survival, proliferation, differentiation, and persistence of the cardiac progenitor cells after transplanted into an immunocompromised mouse model of myocardial infarction. Strikingly, transplant of Pim-1-expressing progenitor cells significantly improved myocardial healing and function of the infarcted heart in 8 wk. The ability to rejuvenate human cardiac progenitor cells ex vivo by Pim-1 modification is highly encouraging to the development of stem-cell therapy for cardiac aging (Mohsin et al. 2013).

## FUTURE PERSPECTIVES ON CARDIAC AGING INTERVENTIONS

The improved understanding on the molecular mechanisms of cardiac aging has led to promising advancements in the development of cardiac aging interventions. Recent studies have shown the potential of different therapeutic approaches to delay or treat cardiac aging, ranging from CR to pharmacologic interventions (rapamycin, enalapril, and SS-31), recombinant protein therapy (IGF-1 and GDF-11), gene therapy (miRNAs), and cardiac stem-cell therapy. However, future studies will be required to evaluate the translational potentials of these interventions.

As a general rule for cardiac aging interventions, short-term treatment(s) beginning at late life will have higher translational potential compared with long-term or life-long treatments. This is particularly relevant to systemic treatments or treatments that target multiple pathways, as a briefer treatment will lower the chances of irreversible side effects. Therefore, it is important to study the kinetics and pharmacodynamics of the treatment to determine the minimal effective dose and duration, as well as the persistence of the treatment to determine the optimum therapeutic regimen.

Another issue for consideration is that there may be gender-specific differences in therapeutic responses. Many of the potential interventions noted above have been tested in only one gender of animals, and, therefore, potential gender-specific differences in beneficial effects remain unknown. For instance, rapamycin provided a greater lifespan extension in female mice at the initial dose (14 ppm) studied by the NIA Intervention Testing Program (Harrison et al. 2009), but a later study showed an improved effect in males at a higher dose, although the lifespan extension benefit was greater in females than males at each dose. This gender difference was associated with a sexual dimorphism of rapamycin levels in blood (Miller et al. 2014).

## CONCLUDING REMARKS

As the elderly population in developed countries is expected to double in the next 25 years, there will be an urgent need for interventions to attenuate or reverse cardiac impairment and the concomitant negative physiological consequences in the elderly. Recent studies show promising results of multiple novel interventions to delay or reverse cardiac aging. More in-depth understanding of the molecular mechanisms of intrinsic cardiac aging and the mechanistic effects of these interventions will be required to guide the development and future translation of these novel therapies to clinical application. Mechanistic insights may also identify other more specific therapeutic targets and provide guidance toward interventions for other age-related pathologies.

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