Significance of Heart Failure Accompanied by Preserved Ejection Fraction and Aging

For thousands of years, if people were lucky enough to survive childhood illnesses and reach adulthood, they had a good chance of living into their 50s and 60s. However, routine survival into the 80s and 90s is a truly new event in human life. As the world’s population ages, the prevalence of age-related diseases is growing dramatically. In the United States, for example, 1 in every 8 Americans is now aged >65 years, and by 2030 the proportion of Americans aged >65 years will reach 19%. Heart failure affects >1% of individuals aged >50 years and increases progressively with age. Thus, with the ongoing steep rise in the world population of elderly individuals, age-related heart failure is certain to become an increasingly prevalent health condition and a leading cause of mortality in the elderly.

Although heart failure traditionally is associated with reduced contractile function of the myocardium, dilation of the left ventricle (LV), and reduced ejection fraction, there is a growing epidemic of heart failure accompanied by preserved ejection fraction (HFpEF). This form of heart failure usually has a normal-sized LV, often but not always with hypertrophy, and is characterized by a global impairment of depressed cardiovascular function. Approximately 50% of patients hospitalized for heart failure have HFpEF, and the mortality risk for these patients is equivalent to those with heart failure accompanied by reduced ejection fraction (≈50% die within 3 years).

Recent studies have defined aging as one factor in the HFpEF epidemic. Echocardiographic studies often reveal normal or near-normal ejection fraction in elderly patients with heart failure, with abnormal diastolic relaxation and LV filling. As observed by Borlaug et al., LV stiffness

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Key Words: aging ■ diastolic dysfunction ■ heart failure, diastolic

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Guest Editor: Michael Zile

Heart Failure With Preserved Ejection Fraction
Molecular Pathways of the Aging Myocardium
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Abstract: Age-related diastolic dysfunction is a major factor in the epidemic of heart failure. In patients hospitalized with heart failure, HFpEF is now as common as heart failure with reduced ejection fraction. We now have many successful treatments for heart failure with reduced ejection fraction, while specific treatment options for HFpEF patients remain elusive. The lack of treatments for HFpEF reflects our very incomplete understanding of this constellation of diseases. There are many pathophysiological factors in HFpEF, but aging appears to play an important role. Here, we propose that aging of the myocardium is itself a specific pathophysiological process. New insights into the aging heart, including hormonal controls and specific molecular pathways, such as microRNAs, are pointing to myocardial aging as a potentially reversible process. While the overall process of aging remains mysterious, understanding the molecular pathways of myocardial aging has never been more important. Unraveling these pathways could lead to new therapies for the enormous and growing problem of HFpEF.

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Review
increases progressively with age despite reduction in arterial load. Abnormal diastolic filling is associated with female sex, obesity, age >65 years, hypertension, renal disease, and diabetes mellitus, suggesting that distinct risk factors and pathological mechanisms underlie these conditions.6,12

An overview of HFpEF with particular emphasis on the clinical aspects will be provided in the accompanying review by Sharma and Kass.17 Here we specifically review molecular insights into age-related contributors to changes in myocardial function, an area of paramount importance, which may also affect globally cardiovascular reserve. Understanding the molecular events in myocardial aging will be essential to develop treatments for the types of heart failure we will see in this century.

### Dissecting the Components of Myocardial Remodeling in Age-Related HFpEF

Aging is an evolutionarily conserved yet poorly understood process that leads to deterioration of many physiological functions over the life-span of an organism.14 Studies have suggested that aging may contribute independently to deterioration of diastolic function.19 Normal cardiac aging is characterized by structural and functional changes. Increased cardiomyocyte size, increased apoptosis with decreased myocyte number, increased collagen deposition, and also functional changes at the cellular level may all contribute to abnormal diastolic function with normal aging.20 The consequences of these changes are an increase in LV diastolic stiffness with aging.19,21 In both the Baltimore Longitudinal Study on Aging and the Framingham Heart Study, LV hypertrophy increased with age, while systolic function was maintained.22 However, while cardiomyocyte hypertrophy occurs in aging human hearts, comorbid diseases such as hypertension also are much more common in the elderly, complicating our understanding of the effects of aging.23 LV diastolic filling rate deteriorates with progressive age,24 and this decline is observed as early as age 20 years in humans.25 By 80 years of age, the reduction in early diastolic filling is as profound as 50%.25 Adult mammalian heart replenishes its cardiomyocyte pool both during physiological aging and in response to injury26–28; cardiomyocyte refreshment, which occurs at a low rate (≈1%/y) in youth, seems to slow even further with advancing age.29 Aging does not seem to affect the low rate of cardiomyocyte apoptosis in normal human hearts.29 In the myocardial extracellular matrix (ECM), aging leads to increased deposition of ECM components, principally collagen, with increased fibril diameter and collagen cross-linking, increased ratio of type I to type III collagen, decreased elastin content, and increased fibronectin.13,30–37 These changes may contribute to exercise intolerance with advancing age, although skeletal muscle function declines with age, as well.13,20

### Models of Cardiac Aging

Cardiac aging in rodents recapitulates many changes observed in humans, with age-dependent increases in LV mass index as well as impaired LV filling.22,38 Studies have documented an age-dependent increase in cardiomyocyte size in wild-type mice,39–41 and age-dependent cardiomyocyte hypertrophy also occurs in rats.42 Studies of rodents suggest multiple potential mechanisms of cardiomyopathy in aging. For example, cardiac angiotensin II levels are higher in senescent animals.43 Adrenergic and cholinergic signaling may also play a role, as mice with disruption of the type 5 adenylyl cyclase are protected from age-related cardiac hypertrophy and fibrosis,44 and mice with reduced function of the choline transporter exhibit age-dependent decreases in fractional shortening and increases in ventricular size and fibrosis.44 Mitochondrial dysfunction likely also contributes, as the aging myocardium exhibits increased mitochondrial protein oxidation and increased mitochondrial DNA mutations.22 In addition to cardiomyocyte hypertrophy, aging mice develop progressive myocardial fibrosis, associated with molecular signatures of immune cell and inflammatory dysregulation.45 Furthermore, cardiomyocyte stability and intercellular mechanical and functional coupling may be perturbed in aged animals because of disruption of junctional adhesion proteins.46

### Age-Related Determinants of Diastolic Dysfunction

The development of heart failure symptoms in patients with diastolic dysfunction has profound implications, as it carries a 60% 5-year mortality prognosis.3 What tips patients into the symptomatic phase is unknown, as patients are known to have impaired diastolic filling before symptoms emerge.5,13,14 Aging may play a fundamental role in modifying both the passive stiffness of the myocardium and the active diastolic relaxation properties of the myocytes.

### Myocardial Interstitial Fibrosis

Interstitial fibrosis is a hallmark of cardiac aging and a major contributor to myocardial stiffness.47,48 The myocardial ECM is not just a passive scaffold for tissue architecture but also a dynamic participant in cellular signaling.20,45 Studies have shown more than doubling of ECM content in the myocardium of senescent rats.41,47,48 Not only is the collagenous weave thicker, but also increased cross-linking among the collagen filaments confers greater rigidity to the myocardium.20 Fibroblasts are the principal cells secreting ECM components, including collagen, fibronectin, and laminin. In aging as well as under a wide range of hypertrophic stimuli, fibroblasts undergo activation and phenotypic transformation to myofibroblasts, which are characterized by expression of the contractile protein α-smooth muscle actin.45,49,50 Myofibroblasts
control ECM composition by regulating the secretion and activity of proteolytic enzymes, including members of the family of matrix metalloproteinases (MMPs) and their inhibitors plasminogen activator inhibitor 1 and tissue inhibitors of MMPs (TIMPs). Fibroblasts are under the active control of multiple signaling hormones and cytokines, and the profibrotic neurohormonal cascades relevant to cardiac aging are discussed below.

Transforming Growth Factor-β Signaling Pathway
Transforming growth factor-β (TGF-β) is one of the most extensively studied fibroblast-activating growth factors. It mediates myofibroblast transformation as well as transcriptional suppression of the MMPs, thus tipping the proteolytic balance toward net matrix accumulation. Brooks et al studied heterozygous TGF-β (+/−)-deficient mice which at 24 months of age exhibited decreased myocardial fibrosis with a total of 4% interstitial collagen as opposed to 10% observed in wild-type mice. The loss of 1 TGF-β allele also contributed to improved myocardial compliance and performance. Blocking TGF-β activity through administration of a neutralizing antibody attenuates diastolic dysfunction in a pressure overload model of cardiac hypertrophy. TGF-β expression is also upregulated by angiotensin II signaling through the angiotensin II receptor type 1 (AT1) receptor. Angiotensin II increases TGF-β1 mRNA and protein expression levels in both cardiomyocytes and fibroblasts. Administration of angiotensin-converting enzyme inhibitors or AT1 receptor blockers ameliorates cardiac hypertrophy and decreases TGF-β1 levels, implicating TGF-β as a mediator of angiotensin II (ATII) effects. Furthermore, Schultz et al. demonstrated that TGF-β1−/− mice (bred with immunocompromised Rag1−/− background to protect from lethality of complete TGF-β1 loss) are protected from the hypertrophic effects of angiotensin II stimulation. Angiotensin II also activates cardiac fibroblast function through signaling via endothelin-1, interleukin-6, and perioxidase. Downstream TGF-β signaling pathways have been extensively studied. TGF-β binds to the constitutively active TGF-β type II receptor at the surfaces of the target cell and subsequently recruits and phosphorylates a type I receptor, also known as TGF-β type I receptor (ALK5). Downstream, Smad2 and Smad3 are activated through phosphorylation by ALK5 and form a complex with Smad4 that translocates to the nucleus and affects gene expression. Non-Smad signalings pathways mediated by TGF-β include Erk (extracellular-signal-regulated kinase), JNK (c-Jun N-terminal kinase), and p38 MAPK (mitogen-activated protein kinase). Although these downstream signaling pathways have not been explored extensively in the context of cardiac aging, their role in profibrotic signaling has been shown in several animal models.

Oxidative Balance
Another important contributor to activation of profibrotic signaling pathways in the aging myocardium is the presence of a positive oxidative balance. Increased levels of reactive oxygen species (ROS) in the senescent myocardium can cause direct TGF-β activation resulting in upregulation of its downstream effector connective tissue growth factor. Indeed, NADPH oxidase–dependent generation of hydrogen peroxide is required for TGF-β1–induced conversion of cardiac fibroblasts to myofibroblasts. Furthermore, scavenging of ROS through mitochondrially targeted catalase expression ameliorates the age-related myocardial fibrosis. Other TGF-β activators include thrombospondin-1 as well as enzymes such as MMP-2, MMP-9, and plasmin which through their proteolytic activity also regulate the kinetics of TGF-β release from the ECM.

Matrix Metalloproteinases
Many of the MMP family of enzymes as well as their inhibitors have been explored in the context of cardiac aging. Epidemiological studies have shown correlations between increasing age and regulation of these enzymes. Aging may produce a shift in the balance between MMPs and TIMPs that ultimately translates into increased matrix accumulation. In a study of healthy subjects with no prior diagnoses of cardiovascular disease, Bonnema et al. showed that age correlates with an increase in MMP-2, MMP-7, and TIMP-1, TIMP-2, and TIMP-4 levels as well as a decrease in MMP-9 plasma levels. Analysis of Framingham subjects has also shown an age-dependent increase in TIMP-1 plasma levels that was related to major cardiovascular risk factors and to indices of LV hypertrophy. Direct genetic evidence from animal models for the specific role of these enzymes in aging-related ventricular remodeling is lacking. Chiao et al. conducted a genetic study in which MMP-9 null mice of all age groups showed no variation in ventricular filling, unlike wild-type senescent mice where impaired diastolic filling occurs with aging. This functional preservation in MMP-9–deficient mice correlates with attenuation of the fibrotic remodeling observed with age.

Titin and Myocyte Stiffness
Titin has been identified as a major molecular determinant of myocyte stiffness. As the largest molecular component of the myocyte structure spanning from Z disk to the M band of the sarcomere, it has been shown to modulate cardiomyocyte passive stiffness through its I-band region, which has spring-like properties that regulate early diastolic recoil and late diastolic resistance to stretch. Titin modulates its stiffness through post-translational modifications, including phosphorylation by protein kinases A, C-α, and G, which decrease its compliance.

In patients with HFpEF, titin is largely hypophosphorylated. A proposed mechanism by the Paulus group postulates that the increased oxidative stress in diastolic dysfunction depletes the NO reserve, thus lowering protein kinase G activity. Furthermore, the abundant ROS generate disulfide bridges that shorten titin’s N2B segment in elderly hypertensive dogs lowered diastolic LV stiffness. Relative hypophosphorylation of the stiff N2B titin isoform ultimately increases resting tension of cardiomyocytes that contributes to the high diastolic LV stiffness observed in failing human hearts, and restoring phosphorylation of the titin N2B segment in elderly hypertensive dogs lowered diastolic LV stiffness. In addition, multiple transgenic mouse models have been created showing that the absence of either the N2B or the PEVK segment, or shortening the tandem immunoglobulin segment, is sufficient to increase myocardial stiffness and cause impaired diastolic filling.
is emerging as an important mediator of diastolic dysfunction, but the effects of aging on titin are incompletely described.

**Calcium Signaling and Active Diastolic Relaxation**

In addition to the above-discussed changes in passive myocardial stiffness, impairment in active diastolic relaxation has been well documented in diastolic dysfunction. Cardiac relaxation occurs when Ca²⁺ reuptake into the sarcoplasmic reticulum occurs through sarcolemmal Na⁺/Ca²⁺ exchanger. Regulation of SERCA channel activity occurs through interaction with the regulatory protein phospholamban. In its unphosphorylated state, phospholamban interacts with SERCA2a and decreases its affinity for Ca²⁺. Phosphorylation of phospholamban by protein kinase A and Ca²⁺/calmodulin-dependent protein kinase disrupts this inhibitory interaction and augments the SERCA pump activity.

Myocardial preparations from experimental models of impaired diastolic function have revealed a decreased rate of intracellular Ca²⁺ decay resulting in prolonged action potential and impaired diastolic filling. Studies have been equivocal correlating expression levels of calcium-handling proteins and aging-related HFpEF. Cain et al and others have shown that human SERCA2a levels correlate inversely with age, whereas Babušková et al and others have shown no changes in SERCA2a protein or mRNA expression levels in senescent murine myocardium compared with young control animals. When SERCA levels are reported together with phospholamban levels, the evidence has suggested decreased SERCA2a/phospholamban ratio with age. Restoring the SERCA/phospholamban balance through gene transfer of the SERCA2a protein in senescent rat myocardium improves age-related diastolic dysfunction.

Conflicting results have been reported regarding protein and mRNA expression levels of ryanodine receptor, L-type Ca²⁺ channels, and Na⁺/Ca²⁺ exchanger. However, more recent evidence suggests the role of post-translational modification of the Ca²⁺-handling proteins in modifying their activity level. Specifically, in multiple separate studies it has been shown that the increased oxidative stress observed in senescent myocardium leads to oxidative damage of the SERCA pump, thus decreasing its Ca²⁺-sequestering activity and prolonging diastolic relaxation. Phosphorylation is another mechanism of post-translational signaling control in Ca²⁺ homeostasis. The aged myocardium has lower responsiveness to β-adrenergic stimulation, and this has been shown to translate into reduced protein kinase A- and Ca²⁺/calmodulin-dependent protein kinase–mediated phosphorylation of phospholamban and ryanodine receptor, thus decreasing Ca²⁺ handling rate.

**Aging-Related Diastolic Dysfunction and Mitochondria**

Both animal and human studies have shown that with aging, mitochondrial DNA accumulates mutations with reported increases as much as 16-fold, and the components of the mitochondrial apparatus function at a decreased level. Vermulst et al showed that mitochondrial DNA deletions play much greater role than point mutations in premature cardiac aging. In a genetic murine model, mice with homozygous mutation of mitochondrial polymerase γ (Polg<sup>null</sup>) exhibit accelerated aging including cardiac senescence features such as cardiac hypertrophy and dysfunction. The loss of mitochondrial polymerase proofreading capacity leads to deficiently functioning components of the cellular energetics machinery that contribute directly to increased oxidative stress. The specific role of mitochondria in myocyte oxidative stress is supported by the finding that overexpression of catalase targeted to mitochondria but not targeted to peroxisomes protects against cardiac hypertrophy, fibrosis, and failure. Dai et al show that mCAT (mitochondrial targeted catalase) mice exhibit significantly attenuated cardiac aging as demonstrated by increased LV myocardial index, myocardial performance index, and E/A ratio on echocardiography. On a histopathologic level, mice with overexpressed mitochondrial catalase exhibit decreased myocardial hypertrophy and interstitial fibrosis as well as =20% life-span extension.

Dysfunctional mitochondria are eliminated by mitophagy, a specialized form of macroautophagy. This process is particularly important in cells like cardiomyocytes where autophagy is the major determinant in protein and organelle turnover. Macroautophagy efficiency progressively declines in cardiomyocytes during aging. As a result, dysfunctional mitochondria that are more prone to release ROS accumulate within aging cardiomyocytes contributing to increased oxidative stress.

The renin–angiotensin–aldosterone system is a driver of mitochondrial dysfunction. Dai et al have shown elevated myocardial levels of angiotensin II in aging hearts. Angiotensin II signals through binding to the angiotensin receptor-1, a Gq-coupled receptor, which has been shown to stimulate the NOX4 (NADPH oxidase 4) isoform of NADPH oxidase on the mitochondrial membrane. ROS generated by NADPH oxidase sets off an ROS-mediated ROS generation propagating a vicious cycle of oxidative stress damaging mitochondrial components, further exacerbating the above cycle. Thus, activation of angiotensin II signaling may promote myocardial aging.

**Molecular Pathways in Age-Related Diastolic Dysfunction**

The NO–cGMP–Protein Kinase G Signaling Axis/Inflammation

Aging is associated with a systemic proinflammatory state, the so-called inflam-m-aging, that may lead to a functional decline in multiple organs even in the absence of a specific disease and can activate signaling cascades leading to myocardial structural and functional remodeling. Indeed, multiple cross-sectional studies show that increasing age is associated with elevated circulating levels of inflammatory markers, including tumor necrosis factor-α, interleukin-6, interleukin-18, monocyte chemoattractant protein-1, soluble ST2 (interleukin 1 receptor-like 1), and pentraxin 3 (Figure 1). An emerging theory for the pathogenesis of HFpEF proposes that a systemic proinflammatory state produced by comorbidities, including aging, causes coronary microvascular endothelial inflammation. This inflammation ultimately results in increased interstitial fibrosis and cardiomyocyte stiffness that contributes to high diastolic LV stiffness and heart failure development. Moreover, the
Inflamed coronary microvascular endothelial cells, as evidenced by the upregulated expression of endothelial adhesion molecules including vascular cell adhesion molecule-1 and E-selectin in myocardial samples from patients with HFpEF,118,126,127 produce ROS.128–134 Increases in ROS can cause reduction in bioavailability of NO for adjacent cardiomyocytes. Reduced NO causes decreases in cGMP levels, which in turn decreases protein kinase G activity in cardiomyocytes. The inflamed endothelium enables binding and translocation of inflammatory cells, further propagating an inflammatory state within myocardium.118 Low protein kinase G activity translates into cardiomyocyte hypertrophy and h ypophosphorylation of titin, thereby increasing stiffness. Stiff cardiomyocytes and increased collagen deposition by myofibroblasts cause diastolic LV impairment.118

Neurohormonal profibrotic signaling through angiotensin II and endothelin is also facilitated by the presence of endothelial inflammation.118,119,120 Counterbalancing the effect of the oxidative stress on cGMP levels with long-term use of sildenafil has shown benefit for diastolic LV function.130 However, in the largest trial studying PDE5 (phosphodiesterase type 5) inhibitors, the RELAX study (Evaluating the Effectiveness of Sildenafil at Improving Health Outcomes and Exercise Ability in People With Diastolic Heart Failure), sildenafil showed no effect on exercise tolerance in the enrolled subjects with HFpEF.135

**Metabolism and Cardiac Aging**

Reducing calorie intake in many species leads to increased longevity and slows the aging effects in key organ systems.136,137 In fact, Doppler studies in humans practicing caloric restriction reveal no changes in systolic function but improved diastolic function.138 In rats, caloric restriction improves calcium handling and diastolic function.139 It was long thought that lower calorie intake leads to diminished metabolism-associated wear and tear by decreasing generation of harmful metabolic by-products, such as ROS. However, further studies have shown the activation of longevity pathways that control genes protective against apoptosis and maladaptive remodeling.140,141

For example, the sirtuins (silent information regulators, SIRTs) as well as the insulin-like growth factor 1/Akt pathways have been identified as key nutrient sensors involved in cardiac and organismal longevity. Conserved from lower organisms, such as yeast, flies, and worms, up to humans, the 7 members of the sirtuin class are diversely positioned in the nucleus and cytosol responding to the cellular energy balance through their NAD+ cofactor.141–148 The characterization of the role of the first member of this class, Sirt1, in cardiac aging has yielded equivocal results. Alcendor et al144 have shown that low (2.5-fold)-to-moderate (7.5-fold) cardiac-specific overexpression of Sirt1 in a transgenic mouse model attenuated age-dependent cardiac remodeling and dysfunction as well as served a cardioprotective role in the presence of oxidative stress (such as paraquat administration) through FOXO-dependent signaling. In contrast, high expression levels (12.5-fold) of Sirt1 increased baseline myocyte oxidative stress, apoptosis, and cellular hypertrophy that reflected in a functional deterioration of the heart.144 In addition, Sirt1 haploinsufficiency ameliorates the extent of cardiac hypertrophy in the presence of a pressure overload stimulus.146 Pillai et al149 and Sundaresan et al150 demonstrated that the prohypertrophic properties of Sirt1 are mediated through cross-talk with the Akt pathway, which participates in cell survival, protein synthesis, and metabolism. Sirt1 deacetylates Akt, thus freeing it to bind and activate PIP3 (phosphatidylinositol (3,4,5)-trisphosphate). Sirt1 also deacetylates PDK1 (phosphoinositide-dependent kinase-1) allowing Akt phosphorylation by this phosphokinase, thus augmenting Akt activity by as much as ≈1000-fold. Sirt1, therefore, plays a dual function in cardiac aging.149–151

Another member of the sirtuin family, Sirt3, is a NAD+- dependent histone deacetylase primarily localized to the mitochondrial membrane that has been shown to regulate pathways in energy metabolism, apoptosis, and ROS synthesis.152–156 Sirt3 knockout mice do not exhibit grossly different phenotype, but microscopic examination reveals premature aging including mitochondrial swelling, cellular hypertrophy, and fibrosis as early as 13 months of age.152–154 Sirt3+−/− mice also demonstrate greater susceptibility to stress such as transverse aortic banding, to which they respond with an exaggerated hypertrophy
and fibrosis. On the contrary, Sirt3 overexpression is cardioprotective in the setting of hypertrophy induced by stimulation with angiotensin II or isoproterenol. Hafner et al proposed a mechanism of Sirt3 to involve prevention of mitochondrial dysfunction by way of decreasing the activity of the mitochondrial permeability transition pore through deacetylation of its regulatory component cyclophilin D. Furthermore, Sirt3 activates FOXO3a-mediated expression of the antioxidant enzymes superoxide dismutase and catalase.

Recent studies have shed light on the cross-talk between Sirt6 and the insulin-like growth factor 1/Akt pathway and its cardiac effects. Sir6+/- mice exhibit the most progerian phenotype of all sirtuin knockout animal models. These transgenic mice are severely hypoglycemic and die within 1 month of age. Sundaresan et al showed that myocardial samples from humans with heart failure as well as from mouse models of cardiac hypertrophy induced by transverse aortic constriction, angiotensin II, or isoproterenol showed markedly reduced Sirt6 levels. Both single- and double-Sirt6 knockout mice develop significant cardiac hypertrophy and dysfunction. The myocardium of these animals exhibits fibrosis, myocyte hypertrophy, and apoptotic and fetal gene expression. Sirt6 overexpression, on the contrary, protects remodeling and dysfunction in the presence of hypertrophic stimuli. Sirt6 is, thus, a major regulator on the crossroads of 2 nutrient-sensing pathways.

Sirt7 is another sirtuin family member, and similar to Sirt6, its downregulation through double-gene knockout in a mouse model leads to myocardial hypertrophy and decreased lifespan. However, much less extensive evidence exists for Sirt 7 and the rest of the sirtuin enzymes on their roles on cardiac aging. The Sirt proteins through their complex interactions with multiple signaling pathways involved in nutrient responses and mitochondrial function are emerging as important therapeutic targets in the context of cardiac aging.

**Novel Discoveries in Cardiac Aging**

**Senescence Marker Protein 30**
Senescence marker protein 30 (SMP30), a 34-kDa protein, ubiquitously expressed in human organs and preserved across species, has recently been identified as another player in cardioprotection in the setting of aging-associated myocardial changes. Misaka et al demonstrated that SMP30 is a marker of cardiac senescence as levels of this protein decrease in the murine myocardium with aging by as much as 40%. SMP30 has been shown to play a role in multiorgan senescence, including brain, lungs, and kidneys. Misaka’s laboratory created an angiotensin II–induced model of cardiac hypertrophy and showed that SMP30 knockout mice exhibit greater myocardial remodeling when exposed to angiotensin II. They also demonstrated that SMP30 deficiency leads to increased myocardial oxidative stress concomitant with increased NADPH oxidase activity. SMP30, therefore, may tip the redox balance in the aging heart.

**Growth Differentiation Factor 11**
The quest for a humoral factor that can rejuvenate aging phenotypes has been long-standing. Recently, growth differentiation factor 11, a member of the activin/TGF-β superfamily, has been identified as a factor that carries the potential to reverse aging-related cardiac remodeling. Heterochronous parabiosis, an experimental procedure whereby 2 animals of different ages are joined together, identified growth differentiation factor 11 as a candidate hormone that controls the aging myocardial phenotype. Further elucidation of how growth differentiation factor 11 fits in the multiple signaling cascades identified to play a role in cardiac aging is pending.

**MicroRNA Signaling**
MicroRNAs are endogenous small noncoding RNAs, 20 to 23 nucleotides in length, which have emerged as important post-translational regulators of numerous cardiovascular processes, from myocardial infarction to cardiac aging. They are characterized by target promiscuity, as a single microRNA is known to target the expression of ≤100 genes by hybridization with complementary sequences on mRNAs and thus triggering their degradation or translational inhibition. Expressional survey of the 17 to 92 microRNA cluster in the aging heart has implicated microRNA-18 and microRNA-19 as potential regulators of aging cardiomyopathy through their targeting of profibrotic pathways involving TGF-β and thrombospordin-1 signaling. Another study conducted in vitro has also shown that microRNA-22 is upregulated with age in mouse fibroblast isolates. The authors identified mimiccan (osteoglycin, described together with TGF-β1 and -β2) as its target under inverse regulation relationship.

More recently, exciting studies have revealed that microRNA-34a (miR-34a) has been implicated in cardiac aging. Predominantly expressed by cardiomyocytes, miR-34a is upregulated in aging mouse hearts as well as in human heart biopsies (2-fold). The role of miR-34a in cardiac aging has been linked to regulation of apoptosis. Using microRNA target prediction tools, Boon et al identified Ppp1r10 (PNUTS) as a downstream target which is downregulated by miR-34a (luciferase assays have shown direct targeting of miR-34a to the 3’ untranslated region of PNUTS). PNUTS has known antiapoptotic effects: it reduces telomere attrition in vitro and DNA damage through the DNA damage response pathways involving CHK2 (checkpoint kinase 2) activation in the presence of TRF2 (telomeric repeat-binding factor 2). MicroRNAs are opening new potential therapeutic frontiers in cardiac aging, but additional studies delineating their biology in aging are needed.

**Telomere Attrition**
Telomere shortening may contribute to functional decline in different tissues, including myocardial tissue. Telomere shortening has been recently identified as a biomarker of lifetime stress, as early as in childhood, and this stress-related telomere shortening could be responsible for accelerated biological aging. Telomere dysfunction-induced cellular phenotypes characterized by proliferative arrest, apoptosis, and senescence may be less relevant considering that genesis of cardiomyocytes occurs at a low rate by the division of pre-existing cardiomyocytes during normal aging. Interestingly, however, telomerases may regulate functional changes in cardiomyocytes. p53 activation induced by telomere dysfunction may directly affect function of mitochondria and metabolism in cardiomyocytes by repressing peroxisome proliferator-activated receptor-γ coactivator-1α and -1β, thus contributing to the development of an aging dysfunctional phenotype.
Future Directions and Therapeutic Implications

In the 21st century, we are seeing our rapidly aging population afflicted with a cardiovascular syndrome that we are ill prepared to face therapeutically, as we simply do not understand the disease. It is common practice to use HFpEF as a term covering a syndrome that is likely a set of diseases of diverse pathophysiology. Careful dissection of the various pathophysiological factors at play in this clinical syndrome is important to design effective therapeutics (Figure 2).

Systemic inflammation could be a common pathway toward diastolic dysfunction. However, recent discoveries of new molecular pathways in aging suggest that we are at the very beginning of understanding the specific role that aging plays in the myocardium. As these new pathways are explored, it raises the exciting possibility that some effects of myocardial aging are potentially reversible.

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None.

References


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