Contribution of Impaired Mitochondrial Autophagy to Cardiac Aging: Mechanisms and Therapeutic Opportunities

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Abstract: The prevalence of cardiovascular disease increases with advancing age. Although long-term exposure to cardiovascular risk factors plays a major role in the etiopathogenesis of cardiovascular disease, intrinsic cardiac aging enhances the susceptibility to developing heart pathologies in late life. The progressive decline of cardiomyocyte mitochondrial function is considered a major mechanism underlying heart senescence. Damaged mitochondria not only produce less ATP but also generate increased amounts of reactive oxygen species and display a greater propensity to trigger apoptosis. Given the postmitotic nature of cardiomyocytes, the efficient removal of dysfunctional mitochondria is critical for the maintenance of cell homeostasis, because damaged organelles cannot be diluted by cell proliferation. The only known mechanism whereby mitochondria are turned over is through macroautophagy. The efficiency of this process declines with advancing age, which may play a critical role in heart senescence and age-related cardiovascular disease. The present review illustrates the putative mechanisms whereby alterations in the autophagic removal of damaged mitochondria intervene in the process of cardiac aging and in the pathogenesis of specific heart diseases that are especially prevalent in late life (eg, left ventricular hypertrophy, ischemic heart disease, heart failure, and diabetic cardiomyopathy). Interventions proposed to counteract cardiac aging through improvements in macroautophagy (eg, calorie restriction and calorie restriction mimetics) are also presented. (Circ Res. 2012;110:1125-1138.)

Key Words: heart senescence ■ mitophagy ■ oxidative stress ■ resveratrol ■ calorie restriction
The prevalence of cardiovascular disease (CVD) increases dramatically with advancing age. More than 80% of cases of coronary artery disease and >75% of cases of congestive heart failure are observed in geriatric patients. The incidence of CVD, including coronary artery disease, congestive heart failure, and stroke, increases from 4 to 10 cases per 1000 person-years in adults aged 45 to 54 years to 65 to 75 cases per 1000 person-years in those aged ≥85 years. CVD is a major cause of chronic disability in the elderly. Notably, in older persons, subclinical CVD is associated with a decline in physical and cognitive function equivalent to >5 years of aging. The disproportionate prevalence of CVD at advanced age is largely attributable to the long-term exposure to cardiovascular risk factors such as hypertension, dyslipidemia, diabetes mellitus, and physical inactivity. In addition, intrinsic cardiac aging, defined as the development of structural and functional alterations during aging, may render the heart more vulnerable to various stressors, which ultimately favors the development of CVD.

One major challenge in the investigation of cardiac senescence is to discern the effects of age per se from those produced by conditions such as hypertension, body composition changes, or diabetes mellitus, which are highly prevalent in late life. However, age-associated alterations in cardiac structure and function also develop in experimental animals, such as the mouse, which are typically exempt from hypertension, diabetes, and atherosclerosis. In addition, longitudinal studies in human cohorts with very low cardiovascular risk profiles (eg, the Baltimore Longitudinal Study on Aging) have shown that advanced age is associated with abnormalities in cardiac performance and structure, such as a decline in early diastolic left ventricular filling and increases in wall thickness, respectively. These observations indicate that the heart undergoes anatomic and functional changes over the course of aging, the interaction of which with CVD-specific mechanisms may eventually result in an excess risk for CVD in late life. Furthermore, the aging heart is characterized by an impaired responsiveness to stress and by a reduced efficiency of endogenous protective mechanisms (eg, ischemic preconditioning and postconditioning), which results in increased vulnerability to injury.

Although the intimate mechanisms involved in cardiac senescence are not fully understood, the progressive accrual of macromolecular oxidative damage over the lifetime is invoked as a major factor. Reactive oxygen species (ROS) are constantly generated within cells by several enzymatic reactions, including those catalyzed by cyclooxygenases, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and xanthine oxidase; however, the bulk of ROS production occurs as a byproduct of mitochondrial oxidative phosphorylation (OXPHOS). Experimental evidence indicates that mitochondrial function decreases over the course of aging, resulting in increased ROS generation, enhanced free radical–inflicted damage, and further mitochondrial decay. In this scenario, the removal of dysfunctional mitochondria through autophagy is crucial for the maintenance of cell viability. The efficiency of this process declines with advancing age, which may be critically involved in heart senescence and in age-related CVD.

In the next sections, mechanisms linking mitochondrial dysfunction and abnormal ROS production to defective mitochondrial autophagy in cardiac senescence will be reviewed. Subsequently, the involvement of mitochondrial dysfunction and altered autophagy in heart diseases common in advanced age (eg, left ventricular hypertrophy, ischemic heart disease, heart failure [HF], and diabetic cardiomyopathy) will be discussed. Finally, interventions aimed at delaying cardiac aging by targeting autophagy will be presented.

Mechanisms and Consequences of Cardiac Mitochondrial Dysfunction in Advanced Age

The Dual Nature of Mitochondria-Generated Oxidants
Mitochondria are essential for cardiomyocyte function and viability. Indeed, the myocardium is a highly energy-demanding tissue, with mitochondria supplying >90% of ATP. Free radicals are constantly generated during mitochondrial respiration. In physiological conditions, 0.2% to 2% of oxygen is converted into superoxide anion (O$_2^-$) mainly at complex I and III of the electron transport chain (ETC). To counteract the burden of ROS production, the mitochondrion is equipped with a multi-leveled defense network comprising detoxifying enzymes and nonenzymatic antioxidants. Within the mitochondrial matrix, manganese-containing superoxide dismutase (MnSOD, SOD2) converts O$_2^-$ into hydrogen peroxide (H$_2$O$_2$), which is further detoxified into O$_2$ and H$_2$O by glutathione peroxidase (Gpx-1) and peroxire-
doxin (Prx-III). Alternatively, $O_2^-$ can be released in the intermembrane space, where it is converted to $H_2O_2$ by copper-zinc--containing SOD (CuZnSOD, SOD1). In addition, $O_2^-$ leaked in the intermembrane space can be scavenged by cytochrome $c$.16

Once merely considered unwanted byproducts ofOXPHOS, mitochondria-derived oxidants are now viewed as essential signaling molecules necessary for the induction of endogenous defense mechanisms that culminate in increased stress resistance (mitochondrial hormesis or mitohormesis).17 In contrast, excessive ROS generation, defective oxidant scavenging, or both have been implicated in the aging process and in the pathogenesis of several chronic degenerative diseases, including CVD.18 The mechanisms responsible for abnormal mitochondrial oxidant generation during aging and the impact of oxidative stress on heart physiology are presented in the next subsections.

Mechanisms of Mitochondrial Free Radical Generation in the Aging Heart

Damaged cardiac mitochondria can release up to 10-fold more $H_2O_2$ than intact organelles.19 Furthermore, in the presence of non–protein-bound redox cycling metals (eg, iron and copper), $H_2O_2$ can be converted into the highly reactive hydroxyl radical (·OH), through the Fenton and Haber-Weiss reactions. In such circumstances, the mitochondrion is exposed to a high burden of oxidative stress, which results in primary damage to its own constituents. It is worth mentioning that mitochondrial iron content increases with aging in rodent postmitotic tissues, including the myocardium, which may exacerbate the extent of oxidative damage in late life.20

Mitochondrial DNA (mtDNA) is especially prone to oxidative damage because of its proximity to the ETC, the lack of protective histones, and a less efficient repair system than in nuclear DNA (nDNA).21 As a result, the level of oxidatively modified bases in mtDNA is several-fold higher than that in nDNA. Moreover, because of the compactness of the mitochondrial genome (ie, lack of introns), each mutation is likely to affect gene integrity and hence protein function.22,23 It follows that mtDNA mutations can lead to the synthesis of defective ETC components, resulting in impairment ofOXPHOS, decreased ATP production, and further ROS generation.22 The vicious circle that originates from ROS-inflicted mtDNA damage represents the main tenet of the mitochondrial free radical theory of aging and is believed to play a central role in the aging process and in the pathogenesis of age-associated degenerative diseases, including CVD.22

Sahin and colleagues24 have recently provided experimental evidence linking the mitochondrial free radical and telomere-shortening theories of aging. These authors used mice with impaired telomere maintenance caused by the targeted deletion of telomerase reverse transcriptase (Tert$^{-/-}$). Tert$^{-/-}$ rodents develop severe telomere dysfunction when backcrossed for ≥3 generations (G4) and display pathologies not only in highly proliferative tissues but also in postmitotic organs, such as the heart. Specifically, G4 Tert$^{-/-}$ mice develop dilated cardiomyopathy during aging, with left ventricular wall thinning and reduced contractile performance. Mitochondria isolated from the heart and liver of G4 Tert$^{-/-}$ mice exhibit reduced mtDNA content, a decline in complex I and IV activity, impaired respiration, and increased ROS generation. These abnormalities are linked to p53-mediated repression of peroxisome proliferator-activated receptor-γ coactivator-1α and -1β (PGC-1α and PGC-1β) and their downstream targets, nuclear respiratory factor-1 (NRF-1) and mitochondrial transcription factor A (TFAM). Hence, age-related telomerase dysfunction might represent a primary instigator of mitochondrial decay, which in turn would lead to decreased bioenergetic efficiency and increased ROS production through sustained p53 activation and further repression of PGC signaling.25

Consequences of Abnormal Mitochondrial Free Radical Generation on Heart Physiology: Evidence From Rodent Models

Elevated levels of oxidative damage to mitochondrial proteins, lipids, and nucleic acids have been detected in the myocardium of old rodents.26–29 The frequency of mtDNA point mutations and deletions is ~3-fold higher in the aged mouse heart than in young adult controls.30 Similarly, the frequency of the common 4977-bp deletion of mtDNA increases during aging in the human heart and is 5- to 15-fold higher in persons >40 years of age than in younger individuals.31 However, the proof of principle that the accumulation of mtDNA damage and subsequent mitochondrial dys- function may be causative for mammalian aging has been provided by the characterization of mice that express a proofreading-deficient mtDNA polymerase-γ (PolG).32,33 These mutants accumulate a high load of mtDNA mutations and deletions and are characterized by the early appearance of many aging-like phenotypes, including cardiac enlargement. Heart mitochondria of PolG mice exhibit abnormal ETC with depressed activity of complex I and IV, reduced ATP production, and accumulation of enlarged, irregularly shaped mitochondria.32 Furthermore, levels of protein carbonyls are increased in cardiac mitochondria from mtDNA mutator mice compared with wild-type rodents.34 PolG mice die prematurely of dilated cardiomyopathy. Severe cardiomyopathy has also been observed in mice expressing a heart-specific proofreading-deficient mtDNA polymerase.35 Remarkably, the PolG heart phenotype, the cardiac mtDNA mutation load, and the extent of mitochondrial protein oxidation are rescued in part by the overexpression of catalase targeted to the mitochondrial matrix (mCAT).34

Further experimental support for the involvement of the mitochondrial vicious cycle in mammalian aging and heart senescence has been provided by the observation that mCAT overexpression extends mean and maximum lifespan and delays the development of cardiac pathology in mice.36,37 The extent of mitochondrial oxidative damage, including mtDNA deletions, and the rate of $H_2O_2$ generation are significantly attenuated in the heart of old mCAT mice compared with age-matched wild-type littermates.36,37 Collectively, studies in mouse models have made a strong argument in favor of the mitochondrial vicious cycle as a contributing factor to cardiac senescence. However, definitive evidence of the involvement of mitochondrial decay in the aging process requires the reciprocal experiment, that is, the generation
of experimental rodents genetically engineered to experience a reduced rate of mtDNA mutations during aging. If these animals lived longer and maintained a youthful heart performance in late life, the contribution of mitochondrial damage to heart senescence would be established conclusively.

The Autophagic Machinery and the Relevance of Mitochondrial Quality Control to Cardiomyocyte Homeostasis

Regardless of the mechanism(s) primarily responsible for mitochondrial decay during aging, mitochondrial quality control is essential for the preservation of cardiomyocyte homeostasis. This task is accomplished through the complex coordination of several processes (reviewed in Tatsuta and Langer38). An intramitochondrial proteolytic system selectively removes damaged proteins. A second line of defense is provided by the dynamic nature of the mitochondrial population. For instance, the functionality of damaged mitochondria can be restored by their fusion with neighboring intact organelles. Finally, severely damaged mitochondria are eliminated through autophagy.

Types of Autophagy

Autophagy is a self-eating process through which cells degrade their own components, recycling amino acids and other building blocks that eventually can be reused.39 Such degradation is performed by lysosomal acid hydrolases. Depending on the pathway along which cellular components are delivered to lysosomes, 3 types of autophagy can be distinguished: macroautophagy, microautophagy, and chaperone-mediated autophagy. Macroautophagy involves the degradation of long-lived proteins and whole cellular organelles through a multistep process (Figure 1).39 Macroautophagy begins with the formation of a double-layered isolation membrane (phagophore) around the molecules and/or organelles to be degraded. The phagophore grows in size and completely engulfs the cargo, forming an autophagosome. The autophagosome subsequently fuses with a lysosome, evolving into an autolysosome, wherein the cargo is digested. LC3 indicates light chain-3; LAMP2, lysosomal membrane-associated protein-2; and SNARE, Soluble N-ethylmaleimide-sensitive factor Attachment protein Receptor; Vps, vacuolar protein sorting.

Figure 1. Schematic representation of the macroautophagic machinery. Macroautophagy begins with the formation of a double-layered isolation membrane (phagophore) around the molecules and/or organelles to be degraded. The phagophore grows in size and completely engulfs the cargo, forming an autophagosome. The autophagosome subsequently fuses with a lysosome, evolving into an autolysosome, wherein the cargo is digested. LC3 indicates light chain-3; LAMP2, lysosomal membrane-associated protein-2; and SNARE, Soluble N-ethylmaleimide-sensitive factor Attachment protein Receptor; Vps, vacuolar protein sorting.

Of the 3 types of autophagy described, macroautophagy is the best characterized in mammalian cells. Starvation is the strongest stimulus of macroautophagy.39,42,43 During nutrient deprivation, macroautophagy breaks down cellular components, generating amino acids, fatty acids, and carbohydrates, which can be harnessed for energy production and for the synthesis of essential cellular molecules. Macroautophagy is also involved in specific cytosolic rearrangements during embryogenesis and postnatal development.44 Furthermore, macroautophagy is induced during viral or bacterial infections,45 in hypoxia,46 and under various stress conditions, including radiation exposure and increased ROS generation.47,48 In these circumstances, macroautophagy is essential for the maintenance of cell homeostasis by its promotion of the removal of damaged components.11 Indeed, impairments in macroautophagy induce premature aging and shorten the lifespan in several organisms.49–51 Conversely, upregulation
of macroautophagy is proposed to be a major mechanism underlying the lifespan-extending properties of calorie restriction (CR). The execution of macroautophagy involves the coordination of a complex molecular machinery, which is described briefly in the next subsection.

The Autophagic Machinery and the Molecular Regulation of Macroautophagy

To date, >35 Atg (AuTophaGy-related) proteins have been identified in yeasts and mammals; however, the precise role each Atg protein plays during autophagy is not fully established. As illustrated in Figure 1, the process of macroautophagy can be divided into discrete steps, namely, induction and nucleation, expansion, fusion, and degradation. The induction phase is mediated by the ULK1-Atg13-FIP200 kinase complex. The regulation of the nucleation stage, which consists of the recruitment of Atg proteins to the phagophore assembly site, is not yet completely understood. However, the vacuolar protein sorting-34 (Vps34), a class III phosphatidylinositol-3-kinase (PI3K), is required for this step. Vps34 associates with Beclin1, the mammalian homologue of yeast Atg6, and subsequently recruits Atg14 and Vps15 (p150) to the preautophagosomal structure. The elongation and expansion of the phagophore membrane require 2 ubiquitin-like conjugation systems involving Atg12 (conjugated to Atg5) and Atg8/microtubule-associated protein 1 light chain-3 (LC3, conjugated to phosphatidyl ethanolamine), along with other Atg proteins such as Atg9 and Atg16. The fusion of the autophagosome with a lysosome relies on canonical cellular fusion machinery that consists of the Rab-SNARE (Soluble N-ethylmaleimide-sensitive factor Attachment protein REnceptor) system and requires the presence of lysosomal membrane-associated protein-2 (LAMP-2) and the UV radiation resistance-associated gene (UVRAG). Finally, the digestion of the cargo is accomplished by lysosomal hydrolases, followed by the transportation of degraded components into the cytoplasm by lysosomal efflux transporters such as Atg22.

With regard to the regulation of macroautophagy, the mammalian target of rapamycin (mTOR) is considered to be a major checkpoint, linking the cellular nutritional state with the level of ongoing autophagy (Figure 2). Under nutrient-rich conditions, mTOR is active and inhibits the ULK1-Atg13-FIP200 complex required for the induction of macroautophagy. Energy deprivation leads to mTOR inactivation and stimulation of AMP-activated protein kinase (AMPK), which both induce macroautophagy. AMPK functions as an energy-sensing kinase and is activated by increases in the cellular AMP to ATP ratio. Under such circumstances, AMPK promotes autophagy by directly activating ULK1 and by relieving the mTOR-mediated inhibition of macroautophagy.

Although macroautophagy might seem to be a random bulk digestion process, evidence is accumulating that intracellular components can be selectively targeted for degradation. For instance, macroautophagy can be specifically directed toward the removal of peroxisomes (pexophagy), endoplasmic reticulum (reticulophagy), and ribosomes (ribophagy). Likewise, mitochondria can be selectively targeted for degradation via mitophagy. The molecular machinery and the regulation of this cellular pathway are outlined in the next subsection.

Mitophagy: A Specialized Form of Macroautophagy

Mitophagy is a highly selective process that can promote the elimination of dysfunctional or unnecessary mitochondria. The loss of mitochondrial membrane potential (∆ψm) represents a major trigger of mitophagy. Indeed, laser-induced photo damage of selected mitochondria inside living hepatocytes results in the rapid dissipation of ∆ψm, followed by the quick removal of depolarized mitochondria through mitophagy. In addition, oxidative damage can lead to the formation of asymmetrical daughter mitochondria characterized by different ∆ψm, with autophagy specifically targeting mitochondria with lower ∆ψm. Apart from the degradation of damaged mitochondria under stress conditions, mitophagy is essential for mitochondrial turnover in the basal state and during cell differentiation, such as the maturation of reticulocytes into mature red blood cells. The occurrence of selective mitophagy in cardiomyocytes has not yet been demonstrated conclusively.

Investigations into the molecular regulation of mitophagy have unveiled several mitophagy-specific proteins. Parkin and Pink1 are believed to play important roles in the selective degradation of damaged mitochondria, at least under certain circumstances. Parkin is a cytosolic E3-ubiquitin ligase that is selectively recruited to dysfunctional mitochondria and assists in their removal by mitophagy. Pink1 is imported into healthy mitochondria through a ∆ψm-dependent process and is degraded by the presenilin-associated rhomboidlike (PARL) protease. The dissipation of ∆ψm results in the accumulation of Pink1 on the mitochondrial surface, leading to the recruitment of Parkin, which ubiquitinates outer membrane proteins, including the voltage-dependent anion channel (VDAC). It is proposed that ubiquitin-tagged mitochondria are targeted directly to autophagic vacuoles through the interaction of ubiquitinated proteins with the autophagosomal marker LC3. In addition, Parkin can ubiquitinate B-cell leukemia-2 (Bcl-2), thereby derepressing Beclin1.

Recent evidence also suggests that the opening of the mitochondrial permeability transition pore (mPTP) may be required for the selective removal of damaged mitochondria. Opening of the mPTP causes a sudden increase of the inner membrane permeability to solutes with molecular weight up to 1500 Da. This results in mitochondrial depolarization, activation of the mitochondrial ATPase (ATP synthase operating in reverse), and swelling and rupture of the outer membrane. The loss of ∆ψm subsequent to permeability transition targets individual mitochondria for degradation. Notably, in cultured cardiomyocytes, starvation-induced macroautophagy is preceded by mitochondrial depolarization. The loss of ∆ψm and the activation of macroautophagy are prevented by cyclosporin A, an inhibitor of the mPTP component cyclophilin D. Furthermore, starvation fails to induce macroautophagy in cyclophilin D–deficient murine cardiomyocytes, whereas in cardiac cells from mice overexpressing cyclophilin D, autophagy is enhanced even under fed conditions. The nicotinamide adenine dinu-
cleotide–dependent deacetylase sirtuin-3 (SIRT3) appears to be critically involved in the control of mPTP by modulation of cyclophilin D. Indeed, in transgenic mice, the loss of SIRT3 activity leads to increased activation of the mPTP in cardiac mitochondria in response to Ca²⁺ increases, hemodynamic stress, and aging.

Similar to the mPTP, the apoptotic proteins Bnip3 (Bcl-2 and adenovirus E1B 19-kDa–interacting protein-3) and Nix (Nip3-like protein X) are thought to trigger selective mitophagy through mitochondrial depolarization. Moreover, Bnip3 may induce mitophagy by competitively disrupting the inhibitory interaction between Bcl-2 and Beclin1. Finally, Nix associates with mitochondrial membranes and directly interacts with LC3.

Although the molecular regulation of mitophagy has not yet been completely elucidated, the mTOR/AMPK pathway is proposed to be a major checkpoint. AMPK, in addition to stimulating mitochondrial removal through autophagy, enhances the activity of sirtuin-1 (SIRT1) and its downstream target PGC-1α, resulting in stimulation of mitochondrial biogenesis (Figure 2). Hence, through the activity of AMPK, mitophagy and mitochondrial biogenesis are coordinately regulated, maintaining a healthy and functional pool of mitochondria in the cell.

### Impaired Macroautophagy: Why and How Dysfunctional Mitochondria Accumulate Within Old Cardiomyocytes

Metabolically, the heart is highly active, and its mitochondria are challenged with a substantial burden of oxidative stress. Moreover, cardiomyocytes are terminally differentiated postmitotic cells with a lifespan of several decades. Hence, the maintenance of a healthy pool of mitochondria and the efficient removal of damaged and potentially harmful organelles are vital for the preservation of cardiomyocyte homeostasis.

Recent evidence indicates that cardiomyocyte macroautophagy becomes impaired during aging. This may result in the accumulation of dysfunctional mitochondria, further exacerbating the metabolic stress on the heart.
in the accumulation within cardiomyocytes of dysfunctional mitochondria that are bioenergetically inefficient and prone to ROS leakage. Indeed, the ultrastructural analysis of myocardium from aged rodents has revealed the presence of enlarged mitochondria, characterized by swelling, loss of cristae, and matrix derangement. Biochemically, these senescent mitochondria exhibit reduced ATP production and increased ROS generation. It is hypothesized that giant mitochondria may progressively displace functional mitochondria over the course of aging. This phenomenon is attributed to a replicative advantage of damaged mitochondria secondary to their partially deleted genome. Alternatively, giant mitochondria may benefit from a survival advantage, being less likely to be autophagocytosed by virtue of their dimensions. Along these lines, the so-called survival of the slowest theory postulates that damaged mitochondria would suffer from less ROS damage on their own membranes because of a reduced respiratory function and would consequently be less targeted for autophagy than intact mitochondria. Thus, the survival of the slowest hypothesis is in apparent contradiction with the widely accepted mitochondrial free radical theory of aging. This phenomenon is attributed to a replicative advantage of damaged mitochondria secondary to their partially deleted genome. Alternatively, giant mitochondria may benefit from a survival advantage, being less likely to be autophagocytosed by virtue of their dimensions. Along these lines, the so-called survival of the slowest theory postulates that damaged mitochondria would suffer from less ROS damage on their own membranes because of a reduced respiratory function and would consequently be less targeted for autophagy than intact mitochondria. Thus, the survival of the slowest hypothesis is in apparent contradiction with the widely accepted mitochondrial free radical theory of aging. However, it must be considered that $\text{O}_2^-$, which constitutes the main free radical generated directly by the ETC, is not reactive enough to cause significant membrane lipid damage unless it becomes protonated into the more reactive perhydroxyl radical ($\text{HO}_2^-$). This conversion may occur at a slower rate in mitochondria with defective ETC, because of the reduced proton gradient, which might translate into a milder mitochondrial membrane oxidative damage.

Both the mitochondrial free radical and the survival of the slowest theories of aging present a major pitfall: within cells, mitochondria frequently fuse with one another to form large syncyta. As a result, a constant mixing of the total cellular mtDNA pool occurs, which disrupts the link between genotype (damaged mtDNA) and phenotype (defective OXPHOS), which is the actual basis of the 2 theories. Nevertheless, the hypothesis linking mtDNA damage, ETC dysfunction, and abnormal ROS generation may still hold true inasmuch as damaged mitochondria are not efficiently broken down because of defective macroautophagy in aged cardiomyocytes. Indeed, advanced age is associated with the accumulation within postmitotic cells of a nondegradable, polymeric, toxic yellow-brown pigment called lipofuscin or age pigment. Lipofuscin occurs results from peroxide-induced Fenton reactions elicited by intralysosomal materials that produce highly reactive hydroxyl radicals (Figure 3). ROS-derived modifications to proteins and lipids cause cross-linking inside lysosomes/autolysosomes, which generates lipofuscin. Peroxides involved in these Fenton reactions can diffuse into lysosomes from cytosolic damaged mitochondria or may originate from autophagocytosed yet undegraded mitochondria. The accumulation of such intracellular garbage eventually overburdens the autophagosomal-lysosomal degradative capacity by acting as a sink for lysosomal hydrolases. It follows that attempts to digest lipofuscin that has accumulated within a growing number of lysosomes eventually result in the incapacity of macroautophagy to keep up with the cell’s needs. This series of events is thought to trigger a vicious circle in which autophagic failure and the accumulation of damaged mitochondria perpetuate each other, which results in further oxidative stress and enhanced lipofuscinogenesis. The collapse of the catabolic machinery will eventually become incompatible with the maintenance of cell homeostasis and survival. This assumption represents the basis of the garbage catastrophe theory of aging, also known as the mitochondrial-lysosomal axis theory of aging.

In addition to removing cellular waste, macroautophagy also provides for the elimination of dysfunctional mitochondria, the persistence of which could lead to the induction of apoptosis. Indeed, mitochondria are a major checkpoint for the integration of apoptotic stimuli. Notably, cardiomyocyte removal through apoptosis increases with advancing age, which, combined with insufficient replenishment by cardiac stem cells, may contribute to age-related heart remodeling. However, whether the increased severity of apoptosis experienced by the aged myocardium is directly attributable to autophagic failure is yet to be established.

Although recent evidence supports the involvement of impaired macroautophagy in the accumulation of abnormal mitochondria within old cardiomyocytes, several research questions remain to be addressed. First, the impact of a dysfunctional mitochondrial-lysosomal axis on cardiac aging needs to be established clearly. For instance, whether manipulation of macroautophagy rescues the premature heart senescence phenotype in animal models characterized by high loads of mtDNA mutation and severe mitochondrial dysfunction is yet to be determined. However, inhibition of macroautophagy via cardiac-specific Atg5 deficiency recently has been shown to induce the early appearance of anatomic and functional features of heart senescence. This was accompanied by biochemical and morphological abnormalities, including decreased mitochondrial respiratory function and accumulation of collapsed mitochondria, respectively. Finally, whether the optimization of macroautophagy preserves cardiomyocyte mitochondrial function and delays cardiac aging in humans is currently unknown.

Mitochondrial Dysfunction and Impaired Regulation of Autophagy Increase the Vulnerability to Injury of the Aged Heart

As discussed previously, evidence from animal models suggests that mitochondrial dysfunction plays a pivotal role in cardiac aging (reviewed in Judge and Leeuwenburgh). According to the mitochondrial-lysosomal theory of aging, the progressive impairment of macroautophagy over the course of aging may represent a primary mechanism responsible for the accumulation of damaged mitochondria within aged cardiomyocytes, resulting in decreased ATP availability, enhanced oxidative stress, and eventually cell dismissal. Hence, the efficient removal of defective mitochondria through macroautophagy is essential for maintaining cardiomyocyte homeostasis and viability in the basal state. Mitochondrial dysfunction is also implicated in the pathogenesis of a host of heart diseases that are highly prevalent in old age, including coronary artery disease, HF, left ventricular hypertrophy (LVH), and diabetic cardiomyopathy. An altered regulation of macroautophagy has been shown to contribute
to the pathogenesis of these conditions, which further supports the relevance of the mitochondrial-lysosomal axis to cardiac physiology.

In the following sections, the role played by cardiomyocyte mitochondrial dysfunction and abnormal regulation of macroautophagy in specific heart diseases especially common in advanced age will be discussed. This overview will highlight the prospect of targeting macroautophagy as a novel means for achieving therapeutic gain in age-related CVD.

Left Ventricular Hypertrophy
Mitochondrial dysfunction is implicated in the pathogenesis of LVH and in the transition from compensated LVH to HF. In cultured cardiomyocytes, hypertrophy induced by angiotensin II, endothelin 1, norepinephrine, tumor necrosis factor-α, or mechanical stress is associated with increased levels of oxidative stress and ROS-mediated activation of several intracellular signaling pathways, including mitogen-activated protein kinases and nuclear factor-κB. In vivo, the development of LVH in animal models of pressure overload is blunted by the administration of antioxidants. Strong support for the involvement of mitochondria-derived ROS in cardiac hypertrophy has been provided by the observation that mCAT mice are resistant to LVH. In contrast, mice overexpressing wild-type peroxisomal catalase (pCAT) accumulate high levels of mitochondrial protein carbonyls and mtDNA deletions while displaying altered macroautophagic regulation. These animals are prone to developing LVH in response to angiotensin II–induced mitochondrial dysfunction. Furthermore, mitochondrial misalignment and aggregation are observed in adult mice with heart-specific deficiency of Atg5 in response to pressure overload induced by thoracic transverse aortic constriction. These animals develop LVH, contractile dysfunction, and heart dilation. Moreover, a reduced autophagic flux with concomitant appearance of clustered mitochondria has been observed in double-transgenic rats harboring human renin and angiotensinogen genes. These rodents develop angiotensin II–induced cardiac hypertrophy and die prematurely of HF. Four weeks of 40% CR increased the activation of cardiomyocyte macroautophagy and mitigated heart macroscopic and ultrastructural remodeling while reducing mortality. These findings suggest that an abnormal regulation of macroautophagy may contribute to the development of LVH, possibly through the accumulation of dysfunctional mitochondria and subsequent increased ROS generation.

Interestingly, chronically enhanced macroautophagy activity may be involved in the transition from stable LVH to HF. Indeed, in Beclin1+/− rodents subjected to aortic banding, the blunted autophagic response is concomitant with reduced left ventricular remodeling. Conversely, overexpression of Beclin1 results in severe pathological remodeling.

Collectively, these findings suggest that macroautophagy may be either beneficial or deleterious in the setting of pressure overload, depending on the circumstances under which it is induced, the extent to which it is stimulated, and the signaling pathways responsible for macroautophagy activation, with the assumption that both an insufficient and an excessive autophagic response are maladaptive. Understanding the optimal level of autophagic activation is mandatory to design treatments that can harness this cellular process to counteract the development of LVH and its transition to HF.

Ischemia/Reperfusion
Oxidative stress plays a central role in the pathogenesis of myocardial damage during ischemia/reperfusion. In this setting, mitochondria contribute to cardiac dysfunction and cardiomyocyte injury via both a loss of metabolic function and an increased generation of oxidants (reviewed in Lesnefsky et al93). Mitochondrial ultrastructural and functional abnormalities in cardiomyocytes develop early during ischemia and progress over its course. The extent of mitochon-
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Macroautophagy is activated in response to myocardial ischemia and promotes cardiomyocyte survival, likely via maintenance of energy production during acute nutrient deprivation. The degradation of proteins and organelles by macroautophagy generates amino acids and fatty acids, which can be used to maintain mitochondrial ATP production and promote survival of cardiac cells. A further means whereby macroautophagy may protect the ischemic myocardium is through the removal of damaged mitochondria. In mouse embryonic fibroblasts, this adaptation is mediated by upregulation of Bnip3 promoted by hypoxia-inducible factor-1 transcription factor activated by low oxygen concentrations.

The recovery of mitochondrial function during the reperfusion phase is largely dependent on the duration of the ischemic insult. After prolonged oxygen deprivation, extensive damage to ETC complexes ensues, which, in conjunction with the concomitant disruption of antioxidant defenses, leads to further ROS generation and additional mitochondrial and cardiomyocyte injury during reperfusion. The combination of oxidative stress and calcium overload can eventually trigger the opening of the mPTP, which results in cardiomyocyte dismissal.

The involvement of mitochondrial dysfunction in myocardial damage during reperfusion would suggest that activation of macroautophagy might be beneficial. Indeed, macroautophagy is upregulated during reperfusion; however, this adaptation may not necessarily be protective. In a study by Hamacher-Brady et al., inhibition of macroautophagy with wortmannin, 3-methyladenine, RNA interference knockdown of Beclin1, or overexpression of dominant negative Atg5 sensitized cultured cardiomyocytes to apoptosis after simulated ischemia/reperfusion. Conversely, enhancement of macroautophagy through rapamycin treatment or Beclin1 overexpression reduced the extent of cardiomyocyte apoptosis in this experimental model. In contrast, Matsui et al. showed that upregulation of macroautophagy during reperfusion is maladaptive and is accompanied by enhanced cardiomyocyte apoptosis and increased infarct size. These authors demonstrated that AMPK is rapidly inactivated during reperfusion, whereas Beclin1 is upregulated. Both the induction of macroautophagy and cardiac injury are significantly attenuated in mice with heterozygous disruption of the Beclin1 gene. Several mechanisms can be invoked to explain the detrimental effects of cardiac macroautophagy during reperfusion. For instance, excessive activation of macroautophagy could culminate in autophagic cell death. In addition, Beclin1 contains a conserved proapoptotic BH3 domain. Thus, overexpression of Beclin1 during reperfusion might result in further deterioration of mitochondrial function and excessive elimination of cardiomyocytes via apoptosis. This eventuality is also promoted by calpain-mediated truncation of Atg5, followed by its translocation to mitochondria, where it interacts with B-cell leukemia-X long (Bel-XL) to trigger permeabilization of the outer mitochondrial membrane.

Further investigations are needed to obtain a clearer understanding of the role of macroautophagy in the context of ischemia/reperfusion. This knowledge is necessary for the design of novel therapeutic strategies that exploit the homeostatic nature of macroautophagy while avoiding the deleterious consequences of abnormal autophagic activation.

Heart Failure

Various stressors, including humoral factors (eg, catecholamines and renin-angiotensin-aldosterone), pressure overload, and toxins, may be responsible for mitochondrial dysfunction and enhanced ROS generation in the failing heart regardless of the cause. HF mitochondria generate larger amounts of O$_2^-$ in the presence of NADH than normal mitochondria. This enhanced ROS production has been associated with increased levels of lipid peroxidation to mitochondrial membranes, decreased mtDNA copy number, reduced abundance of mitochondrial RNA transcripts, and impaired OXPHOS capacity. In addition, oxidative stress directly impacts cardiomyocyte structure and function by activating signaling pathways involved in myocardial remodeling and failure. This suggests the existence of a pathogenetic link between enhanced ROS production, mitochondrial dysfunction, and the development of HF.

Recent evidence also suggests that an altered regulation of cellular quality control mechanisms, including macroautophagy, may contribute to the pathogenesis of HF. For instance, cardiomyocytes isolated from Atg5-deficient mice show an increased sensitivity to $\beta$-adrenergic stimulation compared with wild-type cells. Indeed, 7-day isoproterenol treatment resulted in left ventricular dilation and cardiac dysfunction in Atg5-deficient mice but not in wild-type controls. This suggests that upregulation of macroautophagy protects cardiomyocytes against the detrimental effects of excessive $\beta$-adrenergic stimulation. In contrast, in transgenic mice expressing the human diphtheria toxin receptor in the heart, HF induced by intramuscular injections of diphtheria toxin was accompanied by the appearance of degenerated cardiomyocytes that showed morphological features of autophagic cell death.

Therefore, the question remains as to whether macroautophagy is protective or maladaptive in the context of HF. As observed with regard to pressure overload, the level of macroautophagy may be critical in determining whether this process will be protective or detrimental. Indeed, the upregulation of macroautophagy might represent an attempt to cope with increased levels of mitochondrial dysfunction and cellular damage, with autophagic cell death resulting from the failure of the autophagy-mediated survival machinery. However, the possibility exists that excessive upregulation of...
macroutpahy may be the primary cause of cardiomyocyte dismissal in the failing myocardium, thereby contributing to the deterioration of cardiac performance. Further studies are needed to decipher the role of macroautophagy in mediating either cardioprotection or cardiomyocyte death in the failing heart to properly manipulate this cellular pathway in patients affected by HF.

**Diabetic Cardiomyopathy**

Diabetic cardiomyopathy is a major cause of HF in diabetic patients and develops independent of underlying coronary artery disease. Diabetic cardiomyopathy is characterized by reduced cardiomyocyte contractility, mitochondrial dysfunction, and increased levels of apoptosis. Decreased mitochondrial respiration and reduced expression of OXPHOS components have been observed in the hearts of obese mice with type 2 diabetes mellitus. These alterations are thought to contribute to cardiac dysfunction by diminishing high-energy phosphate reserves, thereby impairing myocardial contractility. In addition to reduced OXPHOS capacity, increased ROS generation has been documented in diabetic mitochondria from diabetic mice. Notably, overexpression of ROS-detoxifying systems (metallothionein, catalase, and MnSOD) reverses mitochondrial dysfunction and cardiomyopathy induced by diabetes, which suggests a central role for mitochondria-derived oxidants in the pathogenesis of diabetic cardiomyopathy.

The inefficient autophagic removal of damaged mitochondria may promote the accumulation of dysfunctional mitochondria in the diabetic heart. Indeed, downregulation of cardiac macroautophagy, secondary to reduced AMPK activity, has been documented in diabetic mice. Ultrastructurally, hearts from these rodents display several morphological aberrations, including aggregation of chaotically distributed mitochondria. Inhibition of AMPK by a cardiac-specific dominant negative AMPK gene further reduced macroautophagy, exacerbated ultrastructural aberrations, worsened cardiac dysfunction, and increased mortality in diabetic mice. In contrast, treatment with metformin significantly enhanced macroautophagy and ameliorated cardiomyocyte ultrastructural abnormalities while preserving cardiac function. Such benefits were not observed in rodents that expressed a dominant negative AMPK, which indicates that cardioprotection by metformin is accomplished through AMPK-mediated upregulation of macroautophagy.

Collectively, these findings suggest that depression of macroautophagy may promote the accumulation of dysfunctional mitochondria in the diabetic myocardium, thereby contributing to the development of diabetic cardiomyopathy. Therefore, interventions that upregulate macroautophagy appear to be promising means to prevent and/or treat this relevant complication of diabetes.

**Macroautophagy as a Therapeutic Target Against Cardiac Aging**

The critical role postulated for mitochondria-driven oxidative damage in cardiac aging and CVD would suggest that the administration of antioxidants might mitigate the burden of cardiomyocyte injury; however, the efficacy of antioxidant supplementation is still a matter of debate. Indeed, most clinical trials failed to show any positive effect of antioxidants on cardiovascular outcomes. Chronic administration of β-carotene, vitamin A, or vitamin E may even increase cardiovascular mortality. Exploitation of the ability of cells to repair or replace oxidatively damaged molecules and organelles represents an appealing alternative against heart senescence and associated pathologies. In this context, interventions aimed at improving mitochondrial turnover through the fine-tuning of macroautophagy (eg, CR, resveratrol administration, and sirtuin pathway activation) could be especially relevant to delay cardiac aging and manage age-related CVD.

CR, defined as a reduction in food intake without malnutrition, is a robust antiaging intervention and the most powerful physiological inducer of macroautophagy. The modulation of the autophagic response represents a primary mechanism underlying the lifespan-extending properties of CR. Indeed, the inhibition of autophagy prevents the antiaging effects of CR in lower organisms. Whether CR also stimulates mitochondrial biogenesis is controversial. CR can induce macroautophagy through different pathways: the insulin-like growth factor-1/insulin signaling pathway, the sirtuin pathway, the AMPK pathway, and the mTOR pathway. These pathways are intimately interconnected, and all play important roles in mediating different aspects of the response. With regard to cardiac aging, Wohlgemuth et al demonstrated that lifelong 40% CR increased the protein expression of Atg7, Atg9, and lipidated LC3-II in the hearts of old rats. More recently, Shimura et al demonstrated that a similar dietary regimen enhanced autophagic flux in the hearts of aged rats through mTOR suppression. These adaptations were associated with reduced lipofuscin accumulation in the myocardium, downregulation of cardiomyocyte apoptosis, decreases in fiber cross-sectional area, and preservation of left ventricular diastolic function. Similar echocardiographic findings have been reported in people in late middle age who were on long-term CR; however, whether these effects were linked with changes in macroautophagy activity was not investigated. Furthermore, it is presently unclear whether the cardioprotective effects of CR are mediated primarily by improvements in autophagy.

Despite the host of health benefits brought about by CR, it is likely that most people will not be able to sustain drastic food restrictions for the long term. Furthermore, persons practicing chronic severe CR may experience several adverse events, including undesired changes in physical appearance, loss of strength and stamina, menstrual irregularities, infertility, loss of libido, osteoporosis, cold sensitivity, slower wound healing, and psychological conditions such as food obsession, depression, and irritability. Moreover, CR may not be advisable in nonobese older persons, given the fact that low body mass index is associated with increased risk of disability and mortality in advanced age. Thus, considerable effort has been directed toward the discovery of drugs that could mimic the effects of CR without requiring food restriction and its detrimental consequences.

The first CR mimetic identified was 2-deoxy-d-glucose, an analog of glucose shown to extend both mean and maximum
lifespan in Caenorhabditis elegans. However, a recent study demonstrated that although it reproduced a CR-like phenotype, chronic administration of 2-deoxy-D-glucose to rats caused cardiotoxicity and increased mortality.128 Promising CR mimetics with autophagy-inducing properties are those that intersect with the critical signaling pathways identified above and include biguanides such as metformin, which targets the AMPK and insulin signaling pathways129, resveratrol, which affects sirtuin activity53; and rapamycin, which interacts with mTOR signaling.130

Resveratrol has been shown to recapitulate the transcriptional profile and some of the physiological changes that develop under CR.131,132 Indeed, both CR and resveratrol supplementation inhibit gene expression profiles associated with cardiac aging in mice.131,132 In addition, resveratrol improved survival and reduced the prevalence of cardiac pathology in mice fed a high-calorie diet.131 Studies in rodents have also shown that resveratrol inhibits cardiomyocyte apoptosis, protects the myocardium against ischemia/reperfusion injury, prevents LVH, improves endothelial function, inhibits platelet aggregation, and reduces inflammation (reviewed in Petrovski et al133). However, although low doses of resveratrol induce macroautophagy, eliciting a preconditioning-like effect, and generate a survival signal in hearts of rats supplemented with resveratrol,134 SIRT1, which targets the AMPK and insulin signaling pathways129; resveratrol, which affects sirtuin activity53; and rapamycin, which interacts with mTOR signaling.130

Conclusions

The optimal regulation of mitochondrial autophagy is critical for the maintenance of cell homeostasis. This is especially true for cardiomyocytes because of their postmitotic nature and their high reliance on mitochondrial oxidative metabolism for energy supply. Over their lifespan, cardiac cells are exposed to a high burden of mitochondria-derived oxidative damage, which cannot be diluted through cell proliferation. This implies that the maintenance of a healthy pool of mitochondria and the removal of damaged organelles are vital for the preservation of cardiomyocyte function and viability. Autophagy serves this critical role for the degradation of damaged organelles while sparing functional mitochondria. Further, some data from model organisms indicate that interventions that upregulate autophagy may produce unrelated and perhaps undesirable effects. Therefore, a deeper understanding of the various actions performed by autophagy-related factors is warranted. Another major caveat is the lack of suitable assays for measuring the ongoing autophagic flux in humans. This limitation makes it extremely challenging to identify the optimal window of autophagic activation to exploit the cardioprotective effects of macroautophagy without disrupting cardiac homeostatic mechanisms. Finally, most data on the effects of pharmacological or behavioral modulation of macroautophagy on cardiac aging and physiology derive from model organisms in which the role of autophagy may differ from what impacts human health, in part because of difficulties in modeling complex human diseases and degenerative processes in experimental settings. Answering these critical research questions will likely provide cardiologists and geriatricians with novel therapeutic means to postpone the degenerative fate of cardiomyocytes and relieve the burden associated with CVD at old age.

Future Directions

The critical role of mitochondrial autophagy in cardiomyocyte physiology suggests that the development of therapeutic interventions that exploit the homeostatic properties of macroautophagy without stimulating its maladaptive effects would be of great value to preserve cardiac function into old age and manage age-related CVD. To accomplish this challenging task, several critical issues need to be addressed. First, the actual contribution of dysfunctional macroautophagy to the pathogenesis of cardiac senescence and CVD remains to be established clearly. In addition, macroautophagy is not just a random degradation process; rather, it is a highly regulated and potentially selective machinery in the service of the cell’s needs. Therefore, it is mandatory to identify specific pathways or substrates of macroautophagy (dysfunctional mitochondria?) the derangements of which are primarily involved in heart senescence. Moreover, the identification of signaling pathways linking mitochondrial dynamics and selective mitophagy is necessary for the development of therapeutic agents that maximize the removal of damaged organelles while sparing functional mitochondria. In this context, it also must be established whether the effects produced by the specific induction of cardiac mitophagy are comparable to those elicited by general macroautophagy. Furthermore, it should be considered that most macroautophagy mediators have multiple functions, which means that their manipulation may produce unrelated and perhaps undesirable effects. Therefore, a deeper understanding of the various actions performed by autophagy-related factors is warranted. Another major caveat is the lack of suitable assays for measuring the ongoing autophagic flux in humans. This limitation makes it extremely challenging to identify the optimal window of autophagic activation to exploit the cardioprotective effects of macroautophagy without disrupting cardiac homeostatic mechanisms. Finally, most data on the effects of pharmacological or behavioral modulation of macroautophagy on cardiac aging and physiology derive from model organisms in which the role of autophagy may differ from what impacts human health, in part because of difficulties in modeling complex human diseases and degenerative processes in experimental settings. Answering these critical research questions will likely provide cardiologists and geriatricians with novel therapeutic means to postpone the degenerative fate of cardiomyocytes and relieve the burden associated with CVD at old age.

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tion of autophagy may be maladaptive and contribute to disease progression.

In summary, the relevance of mitochondrial autophagy to cardiac physiology suggests the possibility that therapeutic interventions targeting this cellular pathway may represent effective means to counteract heart senescence and age-related CVD. Untangling the complexity of autophagic regulation and managing the dual nature of autophagy are major tasks the field of geriatric cardiology is called to pursue.

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Disclosures

None.

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