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Autophagy and cardiac aging

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Abstract

Cardiovascular disease (CVD) is the leading cause of death and the prevalence of CVD dramatically increases with age. Cardiac aging is associated with hypertrophy, fibrosis, inflammation, and decreased contractility. Autophagy, a bulk degradation/recycling system, is essential to maintain cellular homeostasis. Cardiac autophagy is decreased with age, and misfolded proteins and dysfunctional mitochondria are accumulated in the aging heart. Inhibition of autophagy leads to exacerbated cardiac aging, while stimulation of autophagy improves cardiac function and also increases lifespan in many organisms. Thus autophagy represents a potential therapeutic target for aging-related cardiac dysfunction. This review discusses recent progress in our understanding of the role and regulation of autophagy in the aging heart.

Facts

- Cardiac aging is associated with hypertrophy, fibrosis, inflammation, and contractile dysfunction.
- Autophagy declines in the heart during aging in which enlarged and dysfunctional mitochondria and protein aggregates are accumulated.
- Intracellular signaling alterations (e.g., mTORC1, AMPK, and Sirt1) underlie the decline of autophagy in the aging heart.
- Rapamycin and caloric restriction stimulate autophagy and prolong lifespan in many organisms.

Open questions

- How are mitochondrial dynamics and mitophagy regulated in the course of aging?
- How does the NLRP3 inflammasome in the heart contribute to aging-associated cardiac dysfunction through regulation of autophagy?

- What purpose do alterations in metabolic substrate utilization serve in the aging heart?

Cardiac aging: main features and underlying processes

Cardiovascular disease (CVD) is the main cause of morbidity and mortality and the prevalence increases dramatically with age. The prevalence of heart failure in the young adult population (20–39 years old) is less than 1%, but this rises to ~15% among persons 80 years or older [1]. Aging results in progressive deterioration in the structure and function of the heart. More specifically, aging induces hypertrophy, fibrosis, inflammation and contractile dysfunction in the heart (Fig. 1). The heart is a high-energy demanding organ and cardiac tissue is rich in mitochondria, accounting for ~35% of cardiomyocyte volume [2]. With stress, mitochondria become damaged, and release reactive oxygen species (ROS), as well as molecules such as cytochrome *c* which induce necrotic and apoptotic cell death. In the aging heart, there are abnormalities in mitochondrial function and structure: less ATP production, increased ROS generation, enlargement (often referred to as “giant mitochondria”), loss of cristae, matrix derangement and accumulation of mitochondrial DNA (mtDNA) mutations [3, 4]. The two major sources of cellular ROS are the membrane-associated NADPH oxidase (NOX/DUOX enzymes) and the mitochondria. In cardiomyocytes, mitochondria are the major source of ROS. ROS is constantly produced by mitochondria as a by-product of respiration and this is

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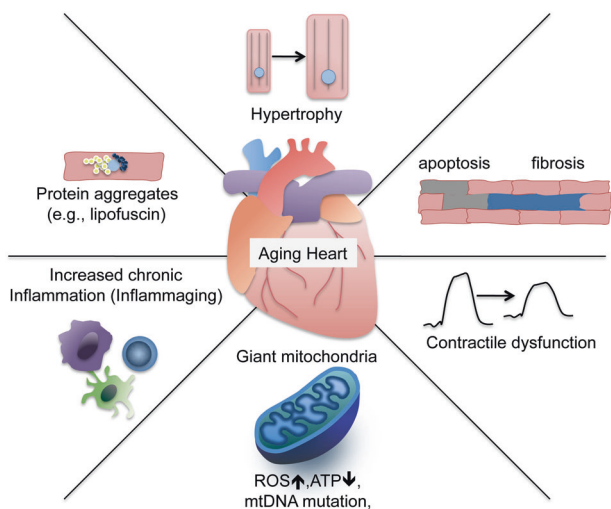


Fig. 1 Characteristics of cardiac aging. Cardiac aging is characterized by functional, structural, cellular, and molecular changes: left ventricular hypertrophy, contractile dysfunction, increased apoptosis and cardiac fibrosis, accumulation of dysfunctional and enlarged “giant” mitochondria, increased chronic inflammation (inflammaging) and accumulation of protein aggregates

counterbalanced by anti-oxidant molecules glutathione and superoxide dismutases. Although low levels of ROS play a physiological signaling role, excessive ROS production is deleterious [5]. Aberrant increase in ROS is mediated by dysfunctional mitochondria and this causes further damage to mitochondria, inducing oxidation-dependent inhibition of mitochondrial proteins, mtDNA mutation and opening of the mitochondrial permeability transition pore and resultant cell death [6]. Oxidative stress also leads to accumulation of protein aggregates, a hallmark of most aging-related diseases. Lipofuscin (aging pigment) [7] is a electron-dense, auto-fluorescent material that accumulates progressively with aging and exhibits cytotoxicity. Advanced glycation end products (AGEs) are produced by glycation, a post-translational modification of proteins, in the cell or in the extracellular space and accumulate with aging [8]. AGEs have been suggested to bind to their receptor (receptor for AGEs: RAGE) to induce oxidative stress, inflammation, and extracellular matrix accumulation. RAGE also functions as a receptor for HMGB1 (High Mobility Group Box 1) released from necrotic cells to initiate inflammatory responses [9]. Inflammation is increasingly recognized as an important contributor to the progression of heart failure through inducing apoptosis, fibrosis and contractile dysfunction [10, 11], and chronic low-grade inflammation is a characteristic of the aging process (inflammaging) [12] (Fig. 1). Elevated interleukin-1 β (IL-1 β), IL-18, and IL-6 expression has been observed in the elderly [13, 14] and recent studies suggest a causative role of inflammation in accelerated aging [10, 15]. IL-1 β and IL-18 are potent pro-inflammatory cytokines, produced by caspase-1 activated by inflammasomes,

including NLR family pyrin domain containing 3 (NLRP3) inflammasome. Aging is associated with an increased frequency of somatic mutations in hematopoietic cells and a recent study demonstrated that clonal expansion of *Tet2* (tet methylcytosine dioxygenase 2, an epigenetic regulator) mutant hematopoietic cells contributes to adverse cardiac remodeling through NLRP3-mediated IL-1 β overproduction [16]. Telomere shortening is also an aging-related genomic change in somatic cells and there exists a correlation between intrinsic epigenetic aging and telomere length [17]. In addition to cell division, factors causing telomere shortening include DNA damage, inflammation, and oxidative stress, thus telomere shortening has been suggested to contribute to cardiac dysfunction with age [16, 18].

Adult cardiomyocytes have a limited capacity to proliferate and regenerate thus cellular quality control is critical in prevention of cardiomyocyte death and cardiac dysfunction. Nutritional and pharmacological interventions that activate autophagy have been demonstrated to increase longevity in organisms ranging from yeast to mammals. This review summarizes recent advances in understanding the role and regulation of autophagy in the aging heart.

Mechanism of autophagy

The term “autophagy (self-eating” in Greek)” was coined by Christian De Duve in 1963 [19], who also discovered the lysosome. Autophagy is a highly conserved and regulated process, and governed by a series of autophagy-related (*ATG*) genes, initially discovered in yeast by pioneering researchers, such as Drs. Yoshinori Ohsumi, Michael Thumm, and Daniel Klionsky [20]. There are three types of autophagy: microautophagy, chaperone-mediated autophagy, and macroautophagy. In this review, we focus on macroautophagy (hereafter referred to as autophagy). Autophagy is a lysosomal degradation process. This self-digestion process was initially considered as a cell death mechanism (type II programmed cell death) and indeed excessive autophagy triggers a specific form of cell death, termed autosis (reviewed by Kriel and Loos in this issue [21]). The molecular and functional interaction between autophagy and apoptosis has been demonstrated, as reviewed by Denton and Kumar in this issue [22]. On the contrary, autophagy can also play an important role in cellular homeostasis under basal conditions, as well as serve as a protective mechanism against stresses by eliminating misfolded proteins and damaged organelles including mitochondria, as well as providing nutrients and energy through degradation of the autophagic cargo [23]. Regulation of autophagy has been suggested to be a potential target for the prevention or treatment of diseases (see review articles in this issue [24–26]). Autophagy consists of several

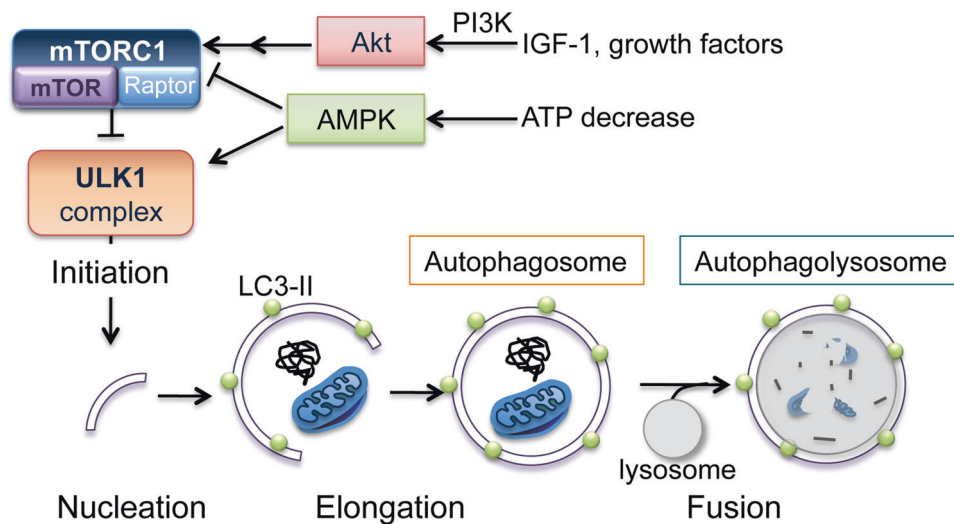


Fig. 2 Autophagy consists of several sequential steps: initiation, nucleation, elongation and fusion. Unc-51 like autophagy activating kinase 1 (ULK1/ATG1) complex plays an essential role in inducing autophagy by initiating the autophagosome formation. ULK1 activity is positively regulated by AMP-activated protein kinase (AMPK) and negatively regulated by mechanistic (mammalian)

target of rapamycin (mTOR) complex 1 (mTORC1). mTORC1 is activated by the PI3K/Akt pathway to inhibit ULK1. Cytoplasmic components and damaged organelles are engulfed by double-membrane autophagosomes which subsequently fuse with lysosomes (autophagolysosomes) for degradation

sequential steps—initiation, membrane nucleation, elongation for autophagosome formation (maturation) and fusion with lysosomes (autophagolysosome) (Fig. 2). Unc-51 like autophagy activating kinase 1 (ULK1/ATG1), a serine/threonine kinase, plays an essential role in the initiation of autophagy. ULK1 activity is negatively regulated by mechanistic (mammalian) target of rapamycin (mTOR). mTOR complex 1 (mTORC1), which contains mTOR and regulatory-associated protein of mTOR (Raptor), phosphorylates and inhibits ULK1, inhibiting autophagy [27, 28]. ULK1 activity is positively regulated by AMP-activated protein kinase (AMPK), a sensor for metabolic suppression. The ULK1 molecular complex containing ATG13, ATG101, and FIP200, in turn, increases activity of VPS34, a class III phosphatidylinositol 3-kinase (PI3K), that forms a molecular complex with several components of the autophagy machinery including Beclin1, VPS15, and ATG14L. Beclin1 undergoes multiple layers of positive and negative regulation, serving as a molecular rheostat, as reviewed by Rubinsztein in this issue [29]. Activation of the VPS34 complex initiates nucleation of the isolation membrane and maturation of autophagosome is regulated by the ubiquitin-like conjugation system, the ATG7, ATG3, and the ATG12-ATG5-ATG16L1 complex mediated LC3-phosphatidylethanolamine (PE) conjugation system. Subsequent fusion of autophagosome with lysosome results in formation of the autophagolysosome where cellular cargo is degraded [30]. A recent study demonstrated that ULK1 regulates not only the initiation step but also fusion with lysosome [31]. For further details regarding the molecular

basis of autophagosome and autophagolysosome formation, please refer to previous review articles [30, 32, 33].

Mitochondrial quality control is fundamentally important in the preservation of cellular integrity thus there exists a process for selective elimination of damaged mitochondria by autophagy (mitophagy) [34]. The means by which damaged (vs. healthy) mitochondria are selected for mitophagic removal is largely attributed to their specific tagging for recognition by autophagosomes. One of the most well-characterized mechanisms of tagging damaged mitochondria is the mitochondrial membrane depolarization-dependent PINK1 (PTEN-induced putative kinase 1)/Parkin pathway. PINK1, a mitochondrial serine/threonine protein kinase, undergoes constant degradation at healthy mitochondria but accumulates upon mitochondrial membrane depolarization and recruits Parkin to damaged mitochondria through phosphorylation of Parkin, mitofusins 2 and ubiquitin [35, 36]. Parkin in turn ubiquitinates mitochondrial proteins, tagging them for mitophagy [34]. Ubiquitinated mitochondrial proteins are recognized by the ubiquitin-binding adaptor proteins including p62/sequestosome 1, optineurin and nuclear dot protein 52 (NDP52), which interacts with LC3-II on the autophagosome membrane, and consequently engulfed and degraded [35, 37]. PINK1 also directly recruits optineurin and NDP52 through phosphorylation of ubiquitin independently of Parkin [38]. Ubiquitin-independent mechanisms also exist, whereby BCL2 Interacting Protein 3 (BNIP3), NIX/BNIP3L, BCL2L13, cardiolipin and FUN14 domain containing 1 (FUNDC1) function as LC3-II receptors, targeting damaged

mitochondria for autophagosomal engulfment and subsequent clearance [39].

Autophagy in the aging heart

Autophagic degradation is established to play a crucial role in preservation of cardiac function against aging. For instance, *Atg5*-deficiency in the mouse heart induces age-related cardiomyopathy [40]. In contrast, *Atg5* overexpression in mice extends lifespan through activation of autophagy [41]. A recent paper also demonstrated that disruption of the Beclin1-Bcl2 complex, a molecular complex that inhibits initiation of membrane nucleation, increases autophagy and inhibits age-induced apoptotic cell death, cardiac hypertrophy and fibrosis, delaying cardiac aging [42]. Mitophagy has also been suggested to be protective against aging. PINK1 knockout (KO) mice show age-dependent impairment of mitochondrial respiration and increased numbers of larger mitochondria in the cortex [43]. Parkin deficient mice accumulate abnormal mitochondria in the heart as they age [44] and Parkin transgenic mice show increased mitophagy and are resistant to cardiac aging, ameliorating the cardiac functional decline and decreasing cellular senescence and inflammation [45]. These seminal studies suggest that stimulation of autophagy can function as an anti-aging mechanism. However, it is generally accepted that autophagy and mitophagy decline with age in many tissues including the heart [40, 42, 45–47]. The mechanisms by which autophagy is reduced with age appear to be highly complex and remain still elusive. In the following section, we discuss the intracellular signaling mechanisms by which autophagic clearance is declined in the aging heart and potential targets to control cardiac autophagy (Fig. 3).

Molecular pathways controlling autophagy in the aging heart

IGF-1/Akt pathway

Akt is activated by growth factors including insulin-like growth factor 1 (IGF-1) and promotes cellular proliferation and growth. In the heart, IGF-1/Akt signaling axis provides strong protection against acute oxidative stress such as ischemia/reperfusion (I/R) and also contributes to development of physiological hypertrophy [48, 49]. However, somewhat paradoxically, it seems evident that IGF-1/Akt pathway negatively regulates aging and lifespan, suggesting that growth-promoting Akt pathway and self-digestive autophagic pathway could serve distinct homeostatic roles in different settings. Plasma IGF-1 levels show an inverse

correlation with a median lifespan in mice [50] and this is also the case in human; low IGF-1 levels predict life expectancy in exceptionally long-lived individuals [51]. Akt is a major upstream activator of mTOR thus it inhibits autophagy (Fig. 3) and activity of Akt is increased in the old mouse heart [47, 52, 53]. Deletion of IGF-1 receptors and suppression of PI3K, an upstream kinase of Akt, prevent cardiac aging in mice with enhanced autophagy [52, 54]. Moreover, ablation of *Akt2*, an isoform of Akt, recovers the level of autophagy in the aging heart and attenuates cardiac aging [55], while Akt overexpression in the heart enhances age-induced decrease in autophagy and exacerbates cardiac aging, such as hypertrophy, fibrosis and contractile dysfunction [53]. Together, these findings suggest that inhibition of IGF-1/Akt signaling promotes autophagy and provides protection against aging-induced cardiac dysfunction.

mTORC1

In line with increased Akt activity, mTORC1 activity has been shown to be increased with age in the mouse heart [47, 53] and in the heart of a mouse model of progeria [56], although it could be sex-dependent and tissue-dependent [57]. Interestingly, gene expression of Raptor, the defining component of mTORC1 (vs. mTORC2) is lower, but that of proline-rich Akt substrate of 40 kDa (PRAS40), an inhibitory binding protein of mTORC1, is higher in non-agenarians, implicating an inverse relationship between mTORC1 pathway and longevity in human [58]. Thus, aberrant activation of mTORC1 contributes to the decreased levels of autophagy in the aging heart (Fig. 3). This is also supported by the findings that genetic and pharmacological inhibition of mTOR increases autophagy and extends lifespan in many organisms and ameliorates cardiac dysfunction with aging [59]. More specifically, rapamycin, a mTORC1 inhibitor, reverses the pre-existing age-dependent cardiac hypertrophy and diastolic dysfunction in mice [60, 61]. Rapamycin treatment in mice also inhibits age-related increases in mitochondrial ROS production, mitochondrial protein lipoxidation and lipofuscin accumulation [60, 62]. These salutary effects of rapamycin could be attributed to enhanced autophagic clearance of damaged proteins and mitochondria, but this requires further experimental clarification since mTORC1 modulates a range of cellular processes. Caloric restriction is another established intervention that extends lifespan in many animal models, in which mTORC1 activity is also decreased [46, 63]. Given that mTORC1 is a key nutrient-sensing kinase, responding to amino acids, as well as glucose [64] to inhibit autophagy under growth conditions, it is likely that mTORC1 inhibition contributes to the anti-aging effects of caloric restriction.

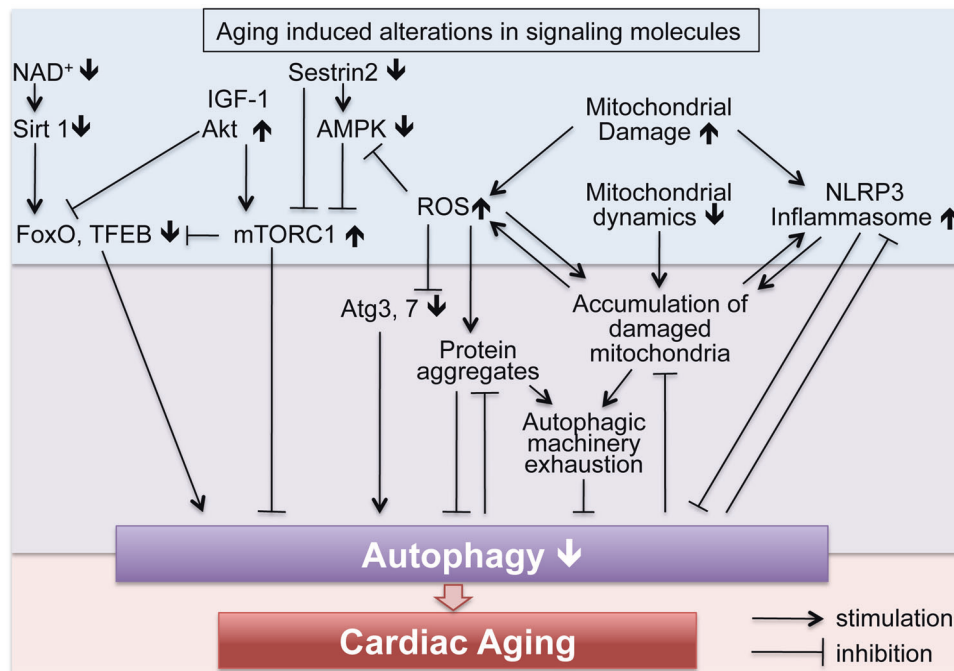


Fig. 3 Regulation of autophagy in the aging heart. Aging inhibits autophagy through multiple mechanisms. Akt is activated by growth factors including insulin-like growth factor 1 (IGF-1). Activity of Akt is increased in the aging heart, leading to activation of mTORC1 and inhibition of autophagy. AMP-activated protein kinase (AMPK) negatively regulates mTORC1 and thus stimulates autophagy. Activity of AMPK is decreased in the aging heart, leading to inhibition of autophagy. Sestrin 2 downregulation contributes to the decrease in AMPK activity. FoxO (Forkhead box O) and TFEB (transcription factor EB) are the transcriptional factors (TFs) to positively regulate autophagy-related, as well as lysosomal gene expression. The activity

of these TFs is diminished in the aging heart through inhibition of sirtuin 1 (Sirt1) activity and through activation of Akt/mTORC1 pathway. Damaged mitochondria release ROS, which lead to accumulation of protein aggregates, inhibition of ATG proteins and cytotoxicity. Impairment of mitochondrial dynamics also participates in accumulation of damaged mitochondria. Activation of the NLRP3 inflammasome negatively regulates autophagy and contributes to accumulation of damaged mitochondria, whereas autophagy negatively regulates the NLRP3 inflammasome by removing danger signals. As a result of these cellular events, autophagic machinery exhaustion and inhibition of autophagy are induced

AMPK

AMP-activated protein kinase (AMPK) is a major sensor for metabolic suppression, activated by reduced cellular ATP levels (increase in AMP/ATP ratio). AMPK negatively regulates the mTORC1 pathway at multiple steps [65]. It phosphorylates and activates tuberous sclerosis 1/2 (TSC1/2), an upstream inhibitor of mTOR, and also phosphorylates Raptor, both resulting in inhibition of mTORC1 [65]. In addition, AMPK directly phosphorylates and activates ULK1, as well as Beclin1 to induce autophagy [65]. Activity of AMPK is decreased in the aging heart [53, 66] and AMPK deficiency exacerbates cardiac aging in mice [67]. AMPK is also activated in the hearts of caloric restriction mice [68]. Metformin, an anti-diabetic drug, activates AMPK and induces autophagy. The mechanism for metformin to activate AMPK remains unclear and controversial. While it was generally accepted that metformin inhibits the respiratory chain complex 1, leading to a drop in cellular ATP levels and thereby activating AMPK [69], a recent study suggests that it acts on the lysosome and

promotes the translocation of LKB1 (liver kinase B1), an upstream kinase of AMPK, onto the surface of lysosome to activate AMPK [70]. Nonetheless, metformin treatment improves healthspan and lifespan in mice [71] and abrogates the aging-induced cardiomyocyte contractile dysfunctions [67].

FoxO and TFEB mediated gene expression

Downregulation of *ATG* genes could contribute to age-related decline in autophagic and mitophagic capacity in the heart. Genome-wide analysis in normal brain aging of human revealed that *ATG5*, *ATG7*, and *BECN1* genes are downregulated with age [72]. Forkhead box O (FoxO) and transcription factor EB (TFEB) are the prominent transcriptional factors to positively regulate autophagy-related, as well as lysosomal gene expression [73, 74]. FoxO1 and FoxO3 regulate autophagic genes such as *ULK1*, *LC3*, *Atg5*, *Atg12*, *Becn1*, and *Bnip3* [73, 75, 76]. TFEB regulates autophagy-related genes such as *Atg4*, *Atg9B*, and *LC3* and is also a master regulator of lysosomal biogenesis [73].

Overexpression of TFEB in the heart increases autophagic flux and provides cardioprotection against oxidative stress [77]. A recent study using a nanotechnology-enabled high throughput screen discovered small molecules that activate TFEB and enhance autophagolysosomal activity, ameliorating metabolic syndrome in mice and prolonging lifespan in *C. elegans* [78]. Akt and mTORC1, anti-autophagic kinases activated in the aging heart, inhibit FoxO3 and TFEB, respectively, leading to inhibition of expression of autophagy genes [79, 80] (Fig. 3). Interestingly, recent studies have demonstrated that Akt/FoxO and mTORC1/TFEB pathways interplay to regulate autophagy. Akt phosphorylates and inhibits not only FoxO but also TFEB [81], while CARM1, a co-activator of TFEB, is stabilized through FoxO3 activation leading to TFEB-dependent gene expression [82]. Together, suppression of FoxO and TFEB activity could contribute to age-dependent autophagy decline in the heart and thus activation of these transcriptional pathways would provide anti-aging effect.

Sirtuins

Sirtuins are nicotinamide adenine dinucleotide (NAD⁺)-dependent deacetylases which are highly conserved from bacteria to mammals. There are seven sirtuins in mammals (Sirt1-Sirt7), among which Sirt1 is the most extensively studied mammalian sirtuin. Many studies have suggested that Sirt1 regulates autophagy and longevity [83, 84], although there are some controversies [85]. Sirt1 expression is decreased by age and its upregulation is suggested to be involved in caloric restriction-induced beneficial effects against aging [86, 87]. The level of NAD⁺ is also decreased with age in many organs, because of downregulation of nicotinamide phosphoribosyltransferase (Nampt) [88] and this plays a causal role in suppressing Sirt1 activity and autophagy. SRT1720 and SRT2104, Sirt1 activators, extend lifespan in mice [89, 90] and resveratrol, a bioactive polyphenol in red wine, activates Sirt1 and autophagy [91]. Injection of nicotinamide mononucleotide (NMN), a product of Nampt and precursor of NAD⁺, provides cardioprotection against I/R through Sirt1 activation [92]. Moreover, moderate levels of overexpression of Sirt1 in the heart retards aging of the heart [83]. Mechanistically, Sirt1 deacetylates and activates FoxO family transcription factors (Fig. 3), thereby facilitating autophagy through upregulation of *Atg* genes and *Rab7* [74, 75, 93], a regulator of autophagosome and autophagolysosome maturation [32, 33]. Sirt1 localizes in the nucleus, as well as the cytoplasm, and cytosolic Sirt1 can also increase autophagy [91]. This could be due to Sirt1-mediated deacetylation of ATG proteins including ATG5, ATG7 and ATG8 [94]. The mTOR signaling pathway is also negatively regulated by Sirt1 through its interaction with TSC2 [95]. Altogether, Sirt1 is a promising candidate to

increase autophagy and provide cardioprotection against aging.

Sestrin

Sestrins (Sestrin1-Sestrin3) are conserved stress-inducible proteins that inhibit mTORC1 through activation of AMPK and through the inhibition of GTPase-activating protein toward Rags 2 (GATOR2), a positive regulator of mTORC1 activation at the lysosome [96, 97], thus they are involved in the induction of autophagy (Fig. 3). Loss of *Drosophila* Sestrin (*dSens*) results in age-associated pathologies including mitochondrial dysfunction and cardiac malfunction in *Drosophila*, which are prevented by pharmacological activation of AMPK or inhibition of mTOR [98]. Sestrin2 is shown to increase ULK1 protein expression levels and to induce mitophagy in macrophages [99], suggesting that multiple mechanisms exist by which Sestrin2 enhances autophagy. Sestrin2 is expressed in the heart and Sestrin2 KO hearts show impaired activation of AMPK in response to ischemia and increased cardiac damage induced by I/R [100]. Importantly, Sestrin2 protein expression is decreased in the heart with age and Sestrin2 KO mice show increased sensitivity to ischemic insults while overexpression of Sestrin2 is protective in old mice [101]. Although there is currently no pharmacological activator of Sestrins, physical exercise increases Sestrin2 protein levels and induces autophagy in the skeletal muscle of old mice [102]. Thus Sestrin2 upregulation could enhance autophagy in the aging heart.

ROS dependent inhibition

Increase in damaged mitochondria and/or imbalances between oxidative stress and antioxidant mechanisms leads to ROS accumulation in the cell. Although oxidative stress can oxidize and inhibit mTOR [103], sustained high levels of ROS may lead to exhaustion of the autophagic pathways (Fig. 3) [104]. AMPK activity is also negatively regulated by oxidation [105] which also leads to inhibition of autophagy by releasing inhibition on mTORC1. A recent study demonstrated that ATG3 and ATG7 are oxidized and that this prevents lipidation of LC3, a critical step in autophagosome maturation [106]. Oxidative stress also mediates formation of lipofuscin which is accumulated in the lysosome and impairs lysosomal function, resulting in accumulation of autophagy substrates including damaged mitochondria [7]. This in turn creates a vicious cycle of ROS generation from damaged mitochondria and ROS-induced inhibition of autophagic degradation. Although ROS accumulation has been implicated in a variety of age-related diseases, translation of ROS scavengers into the clinic has not been successful, and it has been suggested

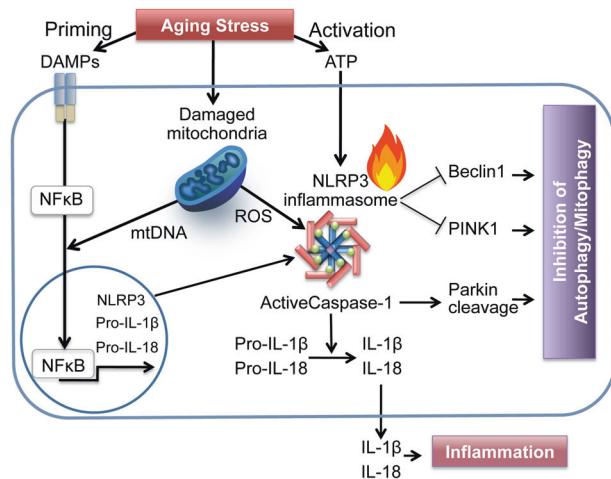


Fig. 4 The NLRP3 inflammasome is primed and activated in response to stress, and induces inflammation and inhibits autophagy. The NLRP3 inflammasome is initially primed by DAMPs (damage associated molecular patterns) including cytosolic mitochondrial DNA (mtDNA) and subsequently activated by stress signals such as extracellular ATP and ROS, leading to caspase-1 activation and production of pro-inflammatory cytokines, IL-1 β and IL-18. NLRP3 negatively regulates autophagy and mitophagy through suppression of Beclin1 and PINK1 and activation of caspase-1 cleaves Parkin and thereby inhibits mitophagy

that targeting ROS scavengers to mitochondria would provide a selective means to prevent the production of pathophysiological ROS [5].

Mitochondrial dynamics

Enlarged mitochondria with membrane and matrix abnormalities accumulate [3, 4] and mitophagy also declines [45, 107, 108] in the aging heart. Mitochondria are highly dynamic organelles that constantly fuse and divide in response to environmental cues. Mitochondrial fusion and fission are functionally related to the mitochondrial quality control mechanisms and critical in maintaining basal cardiac homeostasis as evidenced by the observations that cardiac-specific deletion of dynamin-1-like protein (Drp1), a fission protein or mitofusin-1/2 (Mfn1/2), fusion proteins, causes cardiac dysfunction [109, 110]. Mitochondrial dynamics also play an important role in adaptation to stress conditions, although the effects of stimulation of mitochondrial fusion or fission on cellular survival appear to be context dependent [109, 110]. Interestingly, recent studies, in which fusion and fission are simultaneously inhibited, demonstrated that balanced mitochondrial dynamics but not morphology of mitochondria is critical in quality control of mitochondria (Fig. 3) [111, 112]. More specifically, Mfn1/Mfn2/Drp1 triple KO mouse hearts develop mitochondrial senescence and heart failure from defective mitophagy and accumulation of mitochondria, similar changes to those observed in the aging heart [112]. Despite extensive

research on the role of mitochondrial dynamics in regulating mitophagy, challenges still remain in determining the age-related alterations in molecular signaling regulating mitochondrial dynamics and mitophagy.

NLRP3 inflammasome

The NLRP3 inflammasome comprised of NLRP3, apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase 1 plays a critical role in sensing cellular stress and eliciting inflammation (Fig. 4). The NLRP3 inflammasome is initially “primed” by damage associated molecular patterns (DAMPs) (e.g., extracellular HMGB1, double stranded DNA and cytosolic mtDNA), which leads to upregulation of NLRP3, IL-1 β , and IL-18 mRNA. Subsequently, the NLRP3 inflammasome is assembled and “activated” by stress signals (e.g., extracellular ATP, ROS released from mitochondria) [11]. Thus the NLRP3 inflammasome links mitochondrial and cellular damage to inflammation. The role of the NLRP3 inflammasome in cardiac diseases has been increasingly recognized [113–115] and recent evidence further suggests that NLRP3 inflammasome activation occurs in cardiomyocytes within the heart [113, 116, 117]. For instance, our recent studies have shown that the NLRP3 inflammasome is activated in cardiomyocytes in response to angiotensin-II or pressure overload and that this contributes to recruitment of immune cells, cardiac fibrosis, and ventricular dysfunction [116, 118]. Involvement of inflammasome signaling in cardiac disease has also been suggested by clinical findings using interventions that inhibit IL-1 β function or block the IL-1 receptor [119, 120]. Notably, multiple lines of evidence in non-cardiomyocyte cells have suggested that activation of inflammasome negatively regulates autophagy/mitophagy. NLR family proteins, including NLRP3, bind to Beclin1 and inhibit autophagy [121]. Deletion of NLRP3 leads to higher PINK1 expression leading to elimination of damaged mitochondria and suppression of apoptosis under stress conditions, suggesting an inhibitory role of the NLRP3 inflammasome in PINK1-dependent mitophagy [122]. Deletion of caspase-1, the effector molecule in the inflammasome, increases autophagy and provides cellular protection in macrophages and in neuronal cells [123, 124]. Furthermore, NLRP3 activation triggers mitochondrial damage through caspase-1 activation and this is further amplified by inhibition of mitophagy mediated by Parkin cleavage by caspase-1 in macrophages [125]. These studies clearly highlight the importance of the NLRP3 inflammasome in regulation of autophagy and collectively these findings suggest that there is mutual inhibition between autophagy and inflammasome; autophagy inhibits the NLRP3 inflammasome by removing danger signals including damaged mitochondria, while activation of the

NLRP3 inflammasome inhibits autophagic degradation. This in turn would lead to a vicious cycle that can culminate in further cellular damage.

It has been established that NLRP3 inflammasome links low-grade inflammation to age-related chronic diseases [126, 127]. Deletion of NLRP3 inflammasome enhances healthspan and prevents functional decline in multiple organs, including protection against glucose tolerance, astrogliosis and cataract development [126]. Importantly, NLRP3 inflammasome activity is increased in old mouse hearts [128] and inhibition of RAGE, a receptor for AGEs which is accumulated with age, attenuates adverse effects of angiotensin II through inhibition of NLRP3 inflammasome activity in cardiomyocytes [129]. In addition, recent evidence suggests that NLRP3 is a convergent point of anti-aging interventions. NLRP3 inflammasome is inhibited by rapamycin, metformin, or resveratrol through regulation of mTOR, AMPK, and Sirt1 [130, 131]. Interestingly, nutrients status is shown to modulate the NLRP3 inflammasome in human and mouse. Caloric restriction/fasting induces robust inhibition of the NLRP3 inflammasome [132, 133], while hyperglycemia is associated with upregulation of NLRP3 inflammasome [134]. Thus inhibition of the NLRP3 inflammasome could be involved in caloric restriction induced anti-aging effect. A potent, selective small-molecule inhibitor of NLRP3 (MCC950) has recently been developed [135]. MCC950 blocks NLRP3 activation at nanomolar concentrations [135] and it has been demonstrated that MCC950 reduces ischemic damage in the pig heart [115] and attenuates angiotensin-II induced cardiac fibrosis in mice [116]. Furthermore, MCC950 is shown to induce autophagy and to exert beneficial metabolic adaptations in hearts from high fat and high sugar diet-induced damage [136]. Together, accumulating evidence points to the role of the NLRP3 inflammasome in various cardiac diseases and chronic activation of the NLRP3 inflammasome may contribute to cardiac aging through regulation of autophagy. However, the role of the NLRP3 inflammasome in the heart is still emerging and requires further studies, especially in the aging heart. It would be of importance to determine whether inhibition of the NLRP3 inflammasome in the heart recovers the level of autophagy and prevents cardiac aging.

Concluding remarks

Cardiomyocyte autophagy declines in the course of aging and this directly contributes to cardiac aging. Although our understanding of regulation of autophagy in the heart has greatly improved, it is still not entirely clear how autophagy decreases in the heart with age and there are unsolved questions that warrant future research. First, a more

comprehensive understanding of the molecular signaling changes in various different steps of autophagy in the aging heart is obligatory for identifying precise targets for modulating autophagy therapeutically. Especially, relatively little is known about the regulation of mitophagy in the aging heart. To what extent does the defect of Parkin-dependent vs. that of Parkin-independent mitophagy play a role in aging-associated accumulation of mitochondrial dysfunction? Second, there are only limited data available with regard to autophagy flux in the aging heart especially *in vivo*. It would be critical to determine how autophagy flux is altered with aging. A recent study pointed out the significance of defects in intracellular trafficking of the autophagosome in aging-induced insufficient autophagy [137]. Lysosomal function also declines with age in which altered v-ATPase activity and lysosomal pH dysregulation are implicated as causes [138]. Further clarification of these findings in the aging heart would be beneficial. Third, it is still unclear how changes in cellular metabolic pathways and autophagy interplay in the course of aging in the heart. Alterations in cardiac energy metabolism are induced in pathological conditions and, in the aging heart, there is a shift in myocardial substrate utilization; away from fatty acid utilization to glucose utilization [60, 139, 140]. This is also the case in heart failure induced by pressure overload, whereas the opposite is observed in the heart of diabetics [141, 142]. Notably, an increase in glucose availability in the heart mediated by expression of glucose transporter 1 has been shown to be beneficial in the context of cardiac aging and in the response to ischemia in mice [143]. The protective effect of increase in glucose availability in the heart might not be completely attributed to a switch in substrate utilization [142, 143] and we and others have shown that glycolytic molecules (eg., hexokinase 2 and glyceraldehyde-3-phosphate dehydrogenase) have an ability to facilitate autophagy through inhibition of mTORC1 pathway [144, 145]. Many more studies will be required to delineate the roles of alterations in metabolic pathways in regulation of autophagy in the aging heart. A recent exciting study has demonstrated that oral supplement of the natural polyamine spermidine enhances cardiac autophagy, and induces cardioprotection and lifespan extension in mice [146]. Tissue concentrations of spermidine decline in an age-dependent manner, and thus this suggests the possibility that spermidine-based nutritional supplement could recover the level of autophagy in the aging heart and provide protection against aging.

It is clear that autophagy plays a critical role in maintaining cellular homeostasis and that a decline in autophagy underlies aging-associated cardiac dysfunction. There is ample evidence indicating that activation of autophagy mediates many lifespan extending interventions. Although further understanding of aging-specific alterations in

signaling pathways in the heart will be required, we anticipate that pharmacological targeting of autophagy will provide a novel approach to treating age-related heart diseases.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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