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Autophagy – A key pathway for cardiac health and longevity

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Abstract

As average life expectancy continues to rise in the developed world, age associated pathologies are increasing in prevalence. The hallmarks of cardiac aging include cardiomyocyte loss, fibrosis and hypertrophy, all of which contribute to an increased incidence of cardiac disease. At the molecular level, cellular aging is characterized by increased ROS production, mitochondrial dysfunction and the accumulation of damaged proteins and organelles. Cardiomyocytes and other senescent cell types rely upon autophagy, a lysosome-mediated degradation pathway, to remove potentially toxic protein aggregates and damaged organelles from the cellular milieu. However, increasing lines of evidence point to an age-associated decrease in cardiomyocyte autophagy, with predictably negative consequences for cardiac function and health. Conversely, stimulation of autophagy has been shown to improve cellular health and cardiac function, and to increase lifespan in numerous model organisms. Clearly, autophagy represents a critical pathway for cellular vitality, as well as a promising therapeutic target for the treatment of age related cardiac pathologies. In this review, we will discuss the mechanism of autophagy and its regulation in the cell, the role of autophagy in the aging heart, and how the autophagy pathway might be targeted to improve cardiac health.

The Effect of Aging on Cardiac Structure and Function

With the onset of advanced age, both the heart and vasculature are subject to significant changes in composition and structure, to the detriment of cardiovascular function (Fig. 1). The structural hallmarks of cardiac aging include left ventricular hypertrophy, decline in cardiomyocyte number and fibroblast proliferation, resulting in increased fibrotic tissue area.^{1,2} The net result of these changes is a decrease in LV relaxation and diastolic function, in addition to diminished contractility and ejection fraction. Furthermore, reduced activity of sinoatrial node myocytes coupled with impaired conduction of electrical impulses due to fibrosis produces a decrease in maximal heart rate, further diminishing cardiac output in the aging heart.³

The age-associated decline in cardiac myocyte number is precipitated by multiple factors. Foremost, cardiomyocyte senescence increases with age, with annual rates of turnover decreasing from just 1% in early age, to 0.45% in advanced age.⁴ With such limited capacity for self-renewal, cardiomyocytes lost through standard wear and tear are difficult to replace, making a decrease in number over time inevitable. This is further compounded in advanced

age as cardiomyocytes become more sensitive to stressors, including ROS produced by the mitochondria, leading to increased rates of apoptosis.⁵ In response to the loss of cardiomyocytes, fibroblasts proliferate to fill vacant space with collagen, resulting in fibrosis and a progressive stiffening of the myocardium.

The vasculature is not immune from the effects of aging. Notably, aging arteries increase in both thickness and stiffness, elevating systolic pressure. This is significant, because the health and vitality of the myocardium is inextricably linked to that of the vasculature.⁶

The cumulative effect of the above-listed molecular and structural changes brought about by age on the cardiovascular system is to diminish both systolic and diastolic function and ultimately to decrease cardiac output. Under these circumstances, the heart seeks to make up for the deficit in function via compensatory hypertrophy, with individual cardiomyocytes increasing in size to increase muscle mass as a whole. While this process is initially adaptive, temporarily increasing cardiac output, hypertrophy is not viable in the long term, becoming maladaptive overtime, eventually leading to decompensation and heart failure.⁷

Molecular Mechanisms of Aging in the Heart

The human heart is an extremely hard working organ, consuming in the order of 5–6kg of ATP per day to supply the body with sufficient quantities of oxygenated blood.⁸ Around 95% of the ATP used by the heart is generated by mitochondrial oxidative phosphorylation; for this reason, around 40% of the cytosolic volume in a cardiomyocyte is occupied by mitochondria.⁹ Since mitochondria are the major source of ROS in the cell, cardiomyocytes are at unique risk of ROS accumulation and damage compared to most other cells of the body. The amount of ROS produced by individual mitochondria is inversely linked to the health of the organelle, which declines with age. Mitochondria with defects in the electron transport chain machinery will produce significantly more ROS as a consequence of electron leakage, than their healthier counterparts. Imbalances in levels of ROS producing proteins and antioxidant enzymes further increase ROS levels in aging mitochondria.¹⁰ Accumulated ROS promotes a negative feedback loop in the mitochondria, whereby ROS induced mtDNA damage precipitates mtDNA mutations in key OXPHOS components, which in turn increase ROS generation.¹¹ As a result ROS accumulation is greatly enhanced in aging cells and tissues of the body.

Studies using the mitochondria targeted catalase (mCAT) transgenic mouse model convincingly demonstrate the contribution of mitochondrial-derived ROS to the aging process. Mitochondrial oxidative damage, as measured by mitochondrial protein carbonyls and mtDNA deletions were attenuated in the skeletal muscle and hearts of aged mCAT mice, relative to WT controls.¹² Consequently, cardiac function was vastly improved in aged mCAT mice compared to WT. Left ventricular hypertrophy, fibrosis, and decline in systolic and diastolic function observed in aged WT mice were assuaged by mCAT overexpression in the transgenic group.⁵ Overall, these effects contributed to a 17–21% increase in lifespan in mCAT mice compared to WT.¹²

The contribution of mtDNA damage to the propagation of negative cycles of mitochondrial ROS damage has been demonstrated by the ‘mtDNA-mutator’, or ‘Polg^{m/m}’ mouse model. These mice harbor a homozygous mutation in mitochondrial polymerase gamma, rendering it proofreading deficient and prone to generating point mutations and deletions each time the mitochondrial genome is copied.^{13,14} The lifespan of Polg^{m/m} mice is around half that of WT littermates. The increased mutational burden in Polg^{m/m} mice is responsible for a host of age-associated phenotypes beginning around 9 months, including weight loss, hair loss, kyphosis, and osteoporosis. Mechanistically, one explanation for the advanced aging of tissues and organs in the POLG mice is an increase in apoptosis, driven by mutation of the mitochondrial genome. This would explain why tissues and organs with low rates of cellular renewal, such as the heart, are particularly afflicted in the Polg^{m/m} mouse. Following an early stage compensatory LV hypertrophy, Polg^{m/m} mice progress to cardiomyopathy by 13–14 months of age.¹⁵ This cardiac phenotype was partially rescued by crossing Polg^{m/m} mice with mCAT transgenic mice; once again emphasizing the negative feedback loop between mtDNA mutation and ROS generation.¹⁵ It should be noted, however, that one key shortcoming of both the Polg^{m/m} and mCAT transgenic models, at least for the study of cardiac aging, is that neither is cardiac specific. It is therefore difficult to discount the contribution of mtDNA damage and ROS in other tissues, especially the vasculature, to the cardiac phenotype observed in these mice.

Autophagy and Mitophagy

The efficient removal and replacement of damaged proteins and organelles is essential to maintain cellular homeostasis and to avoid cell death. This is especially true for long-lived cells such as neurons and cardiomyocytes. Additionally, cardiomyocytes are at a greater risk of incurring oxidative damage to their proteins and organelles due to elevated levels of mitochondria derived ROS. Autophagy is an evolutionarily conserved process, by which proteins and organelles are removed from the cell via degradation. This mechanism serves two key purposes; firstly to remove potentially toxic molecules and organelles from the cell. Secondly, autophagy acts as a cellular recycling program during conditions of nutrient deprivation, reclaiming amino acids, lipids and other molecular building blocks liberated from substrates. Autophagy substrates are delivered to the lysosome, wherein lysosomal acidic hydrolases conduct the task of degradation. Three types of autophagy have been classified: macroautophagy, microautophagy and chaperone-mediated autophagy. The finer points of each of these autophagy mechanisms have been reviewed in great depth elsewhere.^{16–18} For the purposes of this review we will primarily focus on macroautophagy, hereafter referred to as ‘autophagy’.

The Mechanism of Autophagy

The following is a deliberately simplistic overview of autophagy. Autophagy is a complex process involving more than 30 genes; while a fine-grained mechanistic description of autophagy is beyond the scope of this review, such information can be found in a number of excellent reviews.^{16–19}

Autophagy consists of three discrete steps: initiation, elongation and maturation/fusion (Fig. 2). Initiation involves the nucleation and formation of a vesicular sac, known as the phagophore. The membrane source from which the phagophore is derived is yet to be determined and a matter of contention in the field. Current evidence points to the ER, mitochondria and plasma membrane as potential contributors.^{20–22} It is also possible that the membrane source may vary depending on the site of autophagosome formation in the cell in addition to the target substrate. Initiation begins with the de-repression of Unc-51 like kinase 1 (ULK1), which is maintained in the inactive, phosphorylated state (pULK1-Ser-757) by mammalian target of rapamycin (mTOR).²³ Inhibition of mTOR allows ULK1 to activate a Class III PI3K complex, consisting of Beclin-1, Vps15 and Vps34. Once active, the PI3K complex initiates phagophore nucleation with assistance from other autophagy proteins.

Elongation, the second step of autophagy, is reliant upon ubiquitin-like conjugation systems. The first of which is the formation of the Atg12-Atg5-Atg16L1 complex. This complex is essential for pre-autophagosomal membrane elongation, and also takes part in LC3-II formation, prior to dissociation from the fully formed autophagosome.²⁴ The second ubiquitin-like reaction facilitates the two-step conversion of LC3 into LC3-II. In the first step, Atg4 cleaves LC3 at its C-terminus to generate LC3-I. Atg3 and Atg7 then promote the lipidation of LC3-I to produce LC3-II.¹⁷ LC3-II is incorporated into both faces of the autophagosomal membrane and is essential for pre-autophagosome elongation and cargo recognition.^{25,26}

Once the pre-autophagic membrane has fused to form an autophagosome, engulfing its cargo in the process, the maturation/fusion phase can begin. Microtubules play a key role here by transporting autophagosomes towards their final destination at the lysosome. In fact, autophagy can be blocked via the disruption of microtubules or by inhibiting dynein.²⁷ Autophagosomes can also fuse with late endosomes on their way towards the lysosome, a process requiring numerous accessory proteins including ESCRT, SNAREs and Rabs.²⁸ However, why endosomal fusion occurs with only some autophagosomes and not others is currently unknown. Finally, the fully mature autophagosomes fuse with the lysosome resulting in the formation of autophagolysosomes. Upon fusion, the contents of the autophagosome, in addition to the inner membrane leaflet will be degraded by lysosomal acidic hydrolases.¹⁹

Mitophagy

Mitophagy is a specialized form of autophagy, dealing in the select removal of unwanted or dysfunctional mitochondria from the cell (Fig. 3). The PINK1 kinase plays a key role in the identification of damaged mitochondria. Under normal conditions, newly synthesized PINK1 is imported into mitochondria where it is subjected to proteolytic cleavage and subsequent degradation.²⁹ Following loss of mitochondrial membrane potential (Ψ_m), PINK1 import is abrogated, accumulating on the outer membrane instead.³⁰ Here, PINK1 recruits the E3 ubiquitin ligase Parkin to the mitochondrial surface, resulting in the ubiquitination of numerous outer membrane protein substrates.³¹ Various adaptor proteins then interact with the ubiquitinated outer membrane proteins, facilitating autophagosomal engulfment of the damaged mitochondria, and degradation at the lysosome.³²

Autophagy in the Aging Heart

There is strong evidence that both autophagy and mitophagy decrease with age in numerous tissues, including the heart.^{33–36} However, the mechanistic underpinnings for the age related decline in autophagy remain unclear and likely complex. One simple yet plausible explanation is that the autophagy machinery is simply overburdened in the aging cell, struggling to keep pace with increased ROS generation and oxidative damage. For instance, a similar situation has been observed in sepsis, in which the rate of autophagic clearance of damaged organelles was outpaced by the extent of sustained insult to hepatocytes.³⁷ Conversely, it is possible that the increase in oxidative stress impairs autophagic activity in cells.^{38,39}

Metabolic and hormonal changes accompanying aging may contribute to lower levels of autophagy. Age-related activation of autophagy suppressors, in addition to inhibition of autophagy activators may also play a role here. For instance, hyperactivation of mTOR, may lead to inhibition of autophagy through mechanisms outlined earlier.⁴⁰ Perhaps the best-known example is the age-related dysregulation of the IGF-1/Akt signaling axis.⁴¹ Mechanistically, this appears to be, in large part, dependent upon members of the FoxO transcription factor family, which are key activators of autophagy gene expression.⁴¹ Aberrant Akt-mediated phosphorylation of FoxO1/3, downstream of IGF-1 receptor activation, promotes nuclear exclusion of FoxO1/3 and thus suppression of autophagy gene expression.^{42,43} Loss of function mutation or inhibition of the IGF-1/Akt pathway significantly increases lifespan in numerous animal models.^{44–46}

Any decrease in the rate of mitophagy is of pressing concern for cardiomyocytes, which are unable to dilute toxic proteins and organelles through cell division. The functional importance of mitophagy has been demonstrated in Parkin-deficient mouse hearts, which exhibit abnormal mitochondrial network morphology at baseline, characterized by fragmented clusters of mitochondria. Following myocardial infarction injury, swollen mitochondria with disrupted cristae accumulate in the infarct border zone of Parkin deficient hearts, contributing to a higher mortality rate in these animals.^{47, 48}

Interestingly, enlarged mitochondria with disrupted cristae are characteristic of both aged and diseased tissues, leading some to hypothesize that the size of these mitochondria may be directly related to their persistence in the cell.^{49–51} Studies have found that mitochondrial fission precedes mitophagic clearance and that elongated mitochondria are protected from degradation.^{52,53} Thus, in this theory, it is assumed that giant mitochondria exceed the engulfment capacity of autophagosomes, whereas normal sized mitochondria continue to be autophagocytosed at the standard rate, resulting in the enrichment of enlarged, dysfunctional mitochondria overtime.⁵⁴ Enlarged mitochondria typically exhibit reduced respiratory function, ATP production and ROS generation. Consequently, in the ‘survival of the slowest’ hypothesis, it is theorized that these mitochondria may be less exposed to sustained ROS induced membrane damage, and therefore less likely to be targeted for removal via PINK1/Parkin-mediated mitophagy.⁵⁵ These two theories are not mutually exclusive, and it’s likely that both contribute to the age associated decline in mitophagy.

The mitochondrial-lysosomal axis theory of aging offers yet another explanation for the decline in autophagy/mitophagy with age. This theory describes the impairment of lysosomal function by the intralysosomal accumulation of lipofuscin, a toxic yellow aggregate, commonly referred to as 'age-pigment'.⁵⁶ Presence of lipofuscin, which can be observed under the microscope by virtue of its autofluorescence, is a hallmark of aging cells. Lipofuscin is formed when ROS-damaged macromolecules, namely proteins, lipids and carbohydrates are cross-linked to produce large aggregates, which are highly resistant to degradation.⁵⁷ The presence of such aggregates strains the degradative resources of the lysosome, impairing autophagosomal/lysosomal function, leading to the accumulation of autophagy substrates including damaged mitochondria. This, in turn, generates a negative feedback loop in which the lysosomal retention of damaged mitochondria contributes to higher levels of oxidative stress and enhanced generation of lipofuscin. Ultimately, these events culminate in a loss of autophagic flux and an inability to maintain cellular homeostasis, compromising cell survival.^{58–60} Accumulation of lipofuscin is especially prevalent in senescent cell types, such as cardiomyocytes, and therefore represents a potential threat to myocardial integrity and function.

Regulation of Autophagy

Sirtuins

Sirtuin 1 (Sirt1) is the mammalian orthologue of silent information regulator 2, an NAD⁺ dependent deacetylase in yeast and drosophila. Numerous studies demonstrate Sirt1, and its orthologues, to be a promoter of both autophagy and longevity in multiple model organisms.⁶¹ The mechanism by which Sirt1 mediates autophagy is not entirely clear. Sirt1 acts in both the nucleus and the cytosol; however, studies using a cytoplasmic restricted Sirt1 mutant suggest that the pro-autophagy effects of Sirt1 are primarily non nuclear.⁶² Sirt1 deacetylates numerous cytosolic targets, including autophagy proteins ATG5, ATG7, and ATG8, favoring an increase in autophagy.⁶³ Importantly, Sirt1 has been shown to retard aging in the myocardium.⁶⁴ Sirt1 stimulates autophagy in fasting hearts via deacetylation of FoxO1 and subsequent upregulation of autophagy mediators.⁶⁵ Furthermore, Sirt1 is protective against ischemia/reperfusion injury of the heart.⁶⁶

Caloric restriction and resveratrol, two long-known promoters of longevity are activators of Sirt1 (Table 1). Caloric restriction upregulates Sirt1 expression in a variety of rodent tissues including brain, liver, kidney and adipose.⁶⁷ Multiple lines of evidence suggest that Sirt1 is in large part responsible for many of the beneficial effects of caloric restriction and resveratrol treatment. Mice deficient in Sirt1 do not show enhanced life span in response to caloric restriction.⁶⁸ Furthermore, in yeast and *C. elegans*, the longevity enhancing effect of CR or resveratrol is abolished following knockdown of *atg* genes.⁶⁹ Taken together, these findings point to the fact that Sirt1 is required for the life span prolonging effects of CR and resveratrol, and that autophagy is key to this process. Sirt1, therefore, represents a promising therapeutic target to increase autophagy and longevity in the myocardium. In addition resveratrol and CR are both well-researched and relatively safe treatment modalities, further favoring Sirt1 as a therapeutic target.

NAD⁺

Sirtuin-mediated decetylation is an NAD⁺ dependent process; therefore, the cellular availability of NAD⁺ is closely linked to the level of protein acetylation, through regulation of sirtuins, as well as autophagic flux and cellular longevity. The cellular concentration of NAD⁺ is a balance between consumption of NAD⁺ by NAD⁺ dependent enzymes and synthesis of NAD⁺ by designated de novo and salvage pathways. NAD⁺ levels decrease with age in many organisms, to the detriment of sirtuin activity and downstream activation of autophagy.^{70, 71} Decreased NAD⁺ levels also impair lysosomal function, further diminishing autophagic flux in the cell.^{71,72} Cardiac specific upregulation of nicotinamide phosphoribosyltransferase (Nampt), a rate-limiting enzyme in the NAD⁺ salvage pathway, increased NAD⁺ content in the heart and was protective against ischemia reperfusion injury.⁷¹ Furthermore, exogenous delivery of nicotinamide mononucleotide (NMN), the product of Nampt in the NAD⁺ salvage pathway, had a similar effect to upregulating Nampt itself.⁷³ NAD⁺ levels were increased in the hearts of NMN treated animals, leading to increased Sirt1 activity and protection against ischemia reperfusion injury.⁷³ Thus NMN treatment represents a promising way to activate autophagy in the heart and improve cellular health and longevity.

AMPK

AMP activated protein kinase (AMPK) is a key nutrient sensor in the cell, as well as a master regulator of metabolic homeostasis and mitochondrial biogenesis.⁷⁴ AMPK is also an important regulator of autophagy in the cell and achieves this via several mechanisms, including inhibitory phosphorylation of mTOR and activation of TSC1/2.⁷⁵ Additionally, AMPK stimulates autophagy through activating phosphorylation of ULK1.⁷⁶ Finally, AMPK enhances NAD⁺ levels in the cell, leading to the activation of Sirt1 and the subsequent deacetylation of Sirt1 targets involved in the activation of autophagy.⁷⁷ Administration of metformin, an AMPK activator, increases lifespan in mice.⁷⁸ In addition, AMPK is activated by thiazolidinediones, exercise and caloric restriction, and is believed to be a key contributor to the therapeutic benefits of these treatments.^{79,80}

mTOR

Mammalian target of rapamycin (mTOR), a serine/threonine protein kinase is an important regulator of nutrient homeostasis. Sitting downstream of IGF-1, sirtuin and AMPK signaling, mTOR integrates inputs from these pathways, thereby acting as a central, nutrient-sensing signaling hub.^{61, 74} mTOR inhibits autophagy through phosphorylation of ULK1 at Ser 757.²³ Either deletion of mTOR, or inhibition with rapamycin extends lifespan in several animal models, and ameliorates age-related damage to the heart.^{81–87} mTOR has a wide variety of cellular functions; however, activation of autophagy is believed to be at least partly responsible for the life extending benefits observed, following the inhibition of mTOR.^{87, 88} IGF-1 mediated stimulation of Akt activates mTOR, thereby inhibiting autophagy. Perhaps unsurprisingly then, loss of IGF-1 in *C. elegans* promotes lifespan extension.⁸⁹ mTOR is readily inhibited by caloric restriction (via AMPK activation), in addition to treatment with rapamycin, the FDA- approved namesake inhibitor of mTOR. mTOR therefore represents an attractive therapeutic target for the promotion of autophagy and extension of lifespan.

Targeting Autophagy

Life-style modification may offer one of the simplest, yet also one of the more effective ways to stimulate autophagy. CR has been shown to extend lifespan and reduce age-related pathologies in multiple animal models. In human trials, a relatively modest 25% reduction in calorie intake significantly improved cardiovascular disease risk, as well as mitochondrial function and health.^{90, 91} While increased autophagy is believed to play a role in these beneficial effects, further research is required to determine the exact contribution. CR has the distinct advantage of simultaneously stimulating multiple upstream activators of autophagy including Sirt1, AMPK and mTOR, making it a particularly attractive and efficacious treatment option. However, a recent study found that neither lifespan nor health of Polg^{m/m} mice were improved by CR, suggesting that this treatment may be rendered ineffective by mitochondrial DNA mutations.⁹²

In addition, voluntary exercise, which has conclusively been shown to reduce the risk of cardiovascular disease in numerous studies, is also an activator of autophagy.⁹³ As with CR, the benefits conferred by exercise to an organism are vast and multifactorial, making it somewhat difficult to pin down the specific contribution of enhanced autophagy. However, one study confirmed that exercise increased autophagic flux in the heart, reducing levels of protein aggregates and alleviating desmin related cardiomyopathy.⁹⁴ CR and exercise share the benefit of being well tolerated in suitable individuals and lack the off-target effects commonly associated with pharmaceutical treatments. Unfortunately, CR or voluntary exercise are viable options for some patients, particularly for those with diminished cardiovascular function. In such cases, pharmacological intervention is a more suitable approach. Furthermore, even in suitable candidates, lifestyle modifications are successful only in the short term for the vast majority of cases, with roughly 95% of individuals unable to sustain these changes beyond three years.⁹⁵

One of the major effects of CR, in relation to autophagy, is to increase the expression and activity levels of Sirt1; therefore, pharmacological activators of Sirt1 may represent a suitable alternative to CR. Resveratrol is one such activator and mimics important outcomes of CR, including protective effects on the myocardium and extension of lifespan, and thus warrants further study.^{69,96}

AMPK activation is also implicated in the beneficial effects of CR. In fact, AMPK may be a particularly appealing target since it also activates Sirt1 via increases in NAD⁺ levels. Furthermore, AMPK also inhibits mTOR, relieving mTOR mediated inhibition of autophagy. The AMPK activator metformin has shown great therapeutic promise in several cardiac disease models, including aging-induced myocardial contractile dysfunction.^{97–100} Since AMPK affects multiple pathways in the cell, further work will be required to identify the exact contribution of autophagy induction to the positive effects of metformin therapy.

Direct inhibition of mTOR via administration of rapamycin has shown encouraging results in the fight against aging. Accumulating reports suggest rapamycin can improve function in aging hearts as well as increase lifespan in general. Again, the contribution of autophagy to these effects remains to be elucidated.

Finally, in a recent study by Eisenberg et al., cardioprotection and extension of lifespan were observed in aged mice given oral supplementation of the polyamine spermidine.¹⁰¹ Mechanistically, spermidine feeding engendered a multitude of positive effects in treated animals including enhanced cardiac autophagy and increased mitochondrial clearance and respiration, in addition to a reduction in systemic blood pressure. Interestingly, the cardioprotective effects of spermidine feeding were lost in mice with cardiac specific deletion of Atg5, clearly demonstrating the importance of autophagy to the efficacy of spermidine treatment. Furthermore, analysis of epidemiological data identified an inverse correlation between dietary spermidine consumption and cardiovascular disease in humans. These results are particularly promising, since treatment may only require minor modification or supplementation of diet.

Conclusion

A decline in cardiomyocyte autophagy is likely a direct contributor to aging in the heart, with negative consequences for cardiac structure and function. However, it is still unclear why autophagy declines with age and whether specific proteins or pathways involved in regulating autophagy are altered with age. Thus, there is a need to further our understanding of the mechanistic underpinnings of autophagy to aid in the design of novel therapeutics to combat aging. To date, most studies on autophagy and mitophagy have been done using immortalized cell lines combined with overexpression of regulators and/or treatments with various toxins or drugs. However, although these systems are valuable to dissect the signaling mechanism, they do not mimic conditions in vivo and have limited physiological relevance to aging. Future studies on autophagy in aging need to prioritize the use of primary cell lines and animal models. Autophagy is a complex process regulated by a number of different pathways; yet, while this makes the study of autophagy more challenging, it also provides a greater potential number of opportunities and targets for therapeutic intervention.

All pharmaceutical therapies currently under investigation as potential autophagy activators also affect other pathways besides that of autophagy; it's therefore difficult to assert what contribution autophagy makes to the positive outcomes arising from these treatments. One goal of future research efforts should be the development of more selective autophagy activators. This will require a deepening of our knowledge of the mechanism of autophagy beyond our current level of understanding. However, many of the off-target effects of activating AMPK, for example, are also of potential benefit for the aging heart and may have a synergistic effect in combination with autophagy activation. It may be the case then, that targeting autophagy in isolation will not be as an effective treatment. Again, further study and greater understanding will be required in order to answer these questions.

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Figure 1 –.
Structural and functional characteristics of the aging heart

Figure 2 –.
Summary of the stages of autophagy

Figure 3 –. Summary of the initiation of mitophagy.

Under basal conditions, PINK1 is subjected to proteolytic cleavage inside the mitochondria. Following a drop in mitochondrial membrane potential, PINK1 accumulates on the outer mitochondrial membrane. Parkin, recruited to the outer membrane by PINK1, ubiquitinates numerous outer membrane target proteins. Adaptor proteins, such as p62, bind to ubiquitinated mitochondrial proteins, facilitating autophagosomal engulfment of the mitochondria.

Table 1

Summary of autophagy targets and effects

Target	Treatment	Effect	Refs
Sirt1	Cardiac specific overexpression	Increased autophagy, protection against I/R injury in mice	63
	Sirt1 deletion	Elevated acetylation of autophagy proteins, decreased autophagy	68
	Caloric restriction	Upregulation of Sirt1 expression, increased autophagy, enhanced lifespan	65–67
	Resveratrol	Increased autophagy, enhanced lifespan	69, 96
NAD⁺	Cardiac specific overexpression of Nampt	Increased autophagy, protective against I/R injury	71
	Nicotinamide Mononucleotide (NMN)	Increased autophagy, protective against I/R injury	73
AMPK	Metformin	Enhanced lifespan, cardioprotection	78, 79, 98–100
mTOR	Rapamycin	Increased autophagy, enhanced lifespan, cardioprotection	84–88