Does autophagy take a front seat in lifespan extension?

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Abstract

This review focuses on the interrelationship between ageing and autophagy. There is a striking similarity between the signalling aspects of these two processes. Both ageing and autophagy involve several of the signalling components such as insulin/IGF-1, AMPK, Ras-cAMP-PKA, Sch9 and mTOR. Ageing and ageing-mediated defective autophagy involve accumulation of lipofuscin. Components of anti-ageing and autophagy include SirTs and FoxOs. Nutritional deprivation or calorie restriction as well as several nutriceuticals including resveratrol, spermidine, curcumin and piperine can enhance autophagy and increase lifespan. Such striking similarities indicate that lifespan is strongly dependent on autophagy.

Keywords: autophagy ● aging ● longevity ● resveratrol ● AMPK ● SirT ● FoxO

Introduction

The existence of a Fountain of youth, a legendary spring that can restore the youth of anyone who drinks its water was believed by a large population in the 16th Century influenced by the Spanish explorer Juan Ponce de León y Figueroa [1]. Such a fountain was mentioned also in the writing of Herodotus some thousands of years ago. In the modern era, even though most may not believe in the existence of such a fountain, it is certain that everyone dreams of living longer. Life expectancy has increased tremendously in recent years—in many developed countries including the USA it is close to 80 years, whereas in underdeveloped countries such as Zambia and Angola life expectancy is below 40 years.

The primary parameter responsible for increased life expectancy is certainly the lifestyle [2, 3]. Stress is likely to be the single most important factor that can reduce life expectancy significantly. Although ageing is certainly a genetically and epigenetically regulated process, modification of the lifestyle is likely to be the best way to increase life expectancy. An existing proof of the concept are the Mormons, a specific group of the World population who live longer than average. Among the many reasons why Mormons live longer is possibly their lifestyle forbidding smoking and allowing regular fasting [2, 3] on the first Sunday of each month [4]. In the later study, 59% of the Mormon fasters were diagnosed with heart disease compared to 67% of the non-fasters (after age, weight and other health conditions were taken into account); the non-Mormon fasters showed same benefits as the Mormon fasters.

During day-to-day activities, accumulation of toxins or damaged particles appearing as misfolded or cross-linked aggregated macromolecules retard the biological and physiological cell functions, acting as sinks for important signalling components of the body [5]. Fasting and calorie restriction (CR) have been proven to stimulate anti-ageing processes and prolonging lifespan by activating detoxifying reactions. Ageing, on the other hand, promotes progressive accumulation of damaged macromolecules and organelles [6, 7].

The question arising is how can fasting perform such a critical task of promoting longevity? Autophagy-an intracellular mechanism for degrading long-lived proteins—probably serves as a boost mechanism for cellular clean-up, preventing accumulation of toxic components and promoting longevity [8]. In fact, suppressing autophagy accelerates accumulation of protein aggregates leading to triggering cell death signals [9]. Interestingly, the ageing...
process involves a co-ordinated program involving a large number of signalling cascades include but not limited to insulin/insulin-like growth factor (IGF)-1, mammalian target of rapamycin (mTOR), JUN NH2-terminal kinase (JNK) and transforming growth factor (TGF) signalling pathways [10–13]. At the same time, the ageing process deactivates several crucial survival protein cascades such as phosphatidylinositol 3-kinase (PI3K)- acutely transforming retrovirus AKT8 in rodent T cell lymphoma (Akt), B cell CLL/Lymphoma-2 (Bcl-2) and/or Sirtuin-Forkhead box (SirT-FoxO) signalling pathways. [14–16]. Autophagy reverses these detrimental signalling processes the same way fasting does [17]. This would tend to suggest that fasting induces autophagy, which in turn would promote anti-ageing signalling. In fact, it is quite true. This review intends to show that anti-ageing effects through lifestyle modification by fasting or chemicals/drugs potentiation of autophagy can clean up the toxic components and induce expression of pro-survival/anti-ageing proteins.

Signalling cascades regulating the ageing process

The large plethora of signalling pathways intersecting at the three major degradative pathways (lysosomal, autophagosomal and proteasomal) is ultimately what decides the balance between ageing and longevity (Fig. 1). Although the death due to ageing for human beings is difficult to correlate because many old people die from other factors such as cardiovascular disease, cancer or diabetes independent of ageing, lifespan and ageing are easy to correlate for lower eukaryotic species. For example, the lifespan of Caenorhabditis elegans can be extended simply by blocking pro-ageing genes [18]. Compared to wild-type C. elegans, mutant species lacking insulin/IGF-1 receptor (Daf-2) live twice their age [19]. Insulin receptor in turn activates class I PI3K-Akt signalling pathway, leading to the inhibition of FoxO—putative player in the human ageing process (Figs. 1 and 2) [20, 21]). This would tend to suggest that inactivation of PI3K/Akt and activation of FoxOs would extend the lifespan of C. elegans [22, 23]. A mitogen-activated kinase member JNK also modulates FoxO and plays a role in the ageing process [24]. The cytokines also appear to be involved as TGF-β signalling seems to be instrumental for autophagy [25]. In addition, mTOR signalling and AMPK (AMP-activated protein kinase) signalling also appear to be involved in the ageing process [26, 27].

Ageing and autophagy

Ageing is a process in which the organisms gradually loose their ability to adapt to the changing environments, become more vulnerable to stress and accumulate damaged products due to overall decrease in the protein degradation time. A growing body of evidence indicates that ageing is regulated by autophagic mechanisms. Autophagy also known as autophagocytosis, is a catabolic process involving the degradation of a cell’s own components
through the lysosomal machinery. It is an evolutionarily conserved intracellular degradative system that operates to maintain cellular homeostasis. In addition, it is responsible for elimination of damaged organelles and intracellular pathogens as well as aberrant or aggregated proteins that cannot be removed via the proteasomal pathway. Autophagy can promote both cell death and cell survival depending on the specific circumstances. Low levels of autophagy in general is considered to be pro-survival up to a certain threshold at which higher level of autophagy cause cellular stress and therefore death described as type II programmed cell death [28, 29]. Based on the selective uptake of cargo destined for degradation, three different forms of autophagy are known to date: 1. **Macroautophagy** in which portions of the cytosol and complete organelles are engulfed by double-membrane structures known as autophagosomes (early autophagic vacuoles), which readily fuse with the lysosome to form single membrane structures autophagolysosomes (late autophagic vacuoles) [28]. By this time, luminal content is degraded and resulting elements are returned into the cytosol to undergo metabolic processes. According to the ability of the autophagosomes to degrade different organelle ‘waste’ material, the intracellular engulfment has been referred as pexophagy (in case of peroxisomes [30]), xenophagy (for intracellular bacteria and viruses [31]) or mitophagy (for mitochondria [32]). Very likely, the future will describe other subtypes of autophagy, possibly ERophagy (for endoplasmic reticulum), ribophagy (for ribosomes) or any other cell organellephagy.

During ageing, age-associated increase in reactive oxygen species (ROS) release and consequent mitochondrial DNA (mtDNA) damage occurs (Fig. 1) [33]. The mitochondria quality control system has been tightly associated with autophagy to segregate damaged, potentially harmful ROSs inside the cell [34]. Hypoxia has been associated with modulation of various cellular processes including autophagy [35] and as a cause of mitochondrial dysfunction and tissue damage [36]—the brain, heart [37, 38] and kidney [39] are all known to be affected in this process. In the brain, for example, autophagy has been implicated as the crucial regulator of the ageing process [40]. Oxidative stress in the irreplaceable post-mitotic neurons can cause epigenetic or specific gene promoters silencing in response to unrepaired DNA damage, therefore evading apoptosis [41]. From worms and flies to mice and human beings, induction of autophagy has been associated with prolonged lifespan and decreased neurodegeneration due to decreased accumulation of ubiquitylated proteins [42, 43]. Attenuation of the mTOR signalling pathway has been shown to extend lifespan in yeast, worms and flies [44]. Rapamycin, an inhibitor of the mTOR pathway extends the lifespan of treated mice by reducing the toxic-aggregate formation in Huntington’s disease model mice [45].

Autophagy in the heart has dual role: under normal conditions, autophagy has a housekeeping role in the turnover of cytoplasmic constituents; failure to do so, may result in defective autophagic degradation (deficiency of lysosomal-associated membrane protein-2 (LAMP-2) causes cardiomyopathy) [46]; cardiomyocytes expressing polyglutamine exhibit increased autophagosomal content eventually causing heart failure [47]; missense mutation in the αβ-crystallin (CryAB) gene increases insoluble CryAB-associated aggregate formation by reducing autophagy causing severe form of desmin-related cardiomyopathy [48]. Autophagy also removes damaged organelles in the heart after cellular distress (hypoxia/ reoxygenation), working against the mitochondrial protein Bnip3 (Bcl-2/adenovirus E1B 19 kD interacting protein) death signalling or apoptosis [49].

Regardless of the organ system involved, CR in general attenuates mitochondrial dysfunction [50] and affects cell adaptation to hypoxia [51]. CR prolongs the lifespan through a silent information regulator 1 (Sirt1) by enhancing autophagy [36]. In the kidney, CR attenuated mitochondrial oxidative damage caused by hypoxia via enhancement of Sirt1 activity. Hypoxia-induced expression of Bnip3 is positively regulated by the hypoxia-inducible factor 1α (Hif1α) and forkhead box O3 (FoxO3) being essential for enhancement of autophagy (Fig. 2).

During the ageing, the efficiency of autophagic degradation reduces resulting in an accumulation of intracellular waste products [52]. Interestingly, the same signalling pathways appear to regulate both ageing and autophagy [53].
2. Microautophagy is a process where engulfment occurs directly by the lysosomal membrane [54]. Although microautophagy similar to macroautophagy is responsible for the disposal of long-lived proteins and organelles, it does not exhibit adaptation to nutritional deprivation. Its distinction from macroautophagy comes from the fact that here small portions of the cytosol are internalized through small lysosomal invaginations for continuous protein degradation even under normal conditions. Studies of this type of autophagy are still limited to yeast, but implications for importance of this process in antigen presentation and the major histocompatibility complex (MHC) do exist [55, 56].

3. Chaperone-mediated autophagy (CMA) is the third type of autophagic processes in which cytosolic proteins containing specific lysosome-targeting motif are recognized by a complex of chaperone protein such as heat shock cognate protein (hsc) 70 and delivered to the lysosomal membranes. After binding to LAMP2A, the protein becomes unfolded followed by translocation into the lysosomes for degradation [57]. Besides the great interplay among the three types of autophagy, CMA in contrast, specifically targets single soluble cytosolic proteins for translocation across the lysosomal membrane and degradation [58, 59]. This specificity is based upon recognition of an amino acid motif on the protein destined for degradation by a cytosolic chaperone complex [60]. Nevertheless, the role of CMA in the turnover of long-lived proteins and intracellular quality control, as well as its role in different diseases has been similar to other types of autophagy.

Almost all proteins targeted for CMA degradation contain a fingerprint pentapeptide KFERQ recognized by the CMA cytosolic chaperone complex, but the same proteins can also contain targeting signals for other proteolytic systems, the ultimate decision for degradation being made upon current cellular conditions or reasons for degradation. A definite ‘ticket’ for degradation of a substrate via CMA is recognition by the hsc70 chaperone/co-chaperone complex [61].

LAMP2A, a single-span lysosomal membrane protein serves to bind and translocate the protein destined for lysosomal degradation. Upon binding, substrate unfolding takes place and lysosomal lumen associated hsc70 protein (lys-hsc70) together with a lysosomal hsp90 protein somehow translocate the cytosolic protein for degradation, which takes only min. [62].

Under normal conditions, CMA has housekeeping and stress-response function, but it can also be activated by accumulation of misfolded or oxidized proteins or starvation itself [63]. Interestingly, under nutritional deprivation LAMP2A receptor levels increase not due to increased expression of this protein, but due to decreased rate of degradation; supply from the pooled LAMP2A on the luminal side of the lysosome regulate the activity of the CMA during starvation [64]. Several pathologic conditions associate with CMA: lysosomal storage diseases (galactosialidosis, mucolipidosis, Danon’s disease), nephropathies (diabetic, acidotic and chronic kidney disease), neurodegenerative disorders (Alzheimer’s, Parkinson’s and Huntington’s disease), oncogenic and immunological diseases [65]. During ageing, the CMA activity also declines mainly as a consequence of decreased levels of LAMP2A and altered turnover of this protein on the lysosomal membrane [66]. A proof of concept was recently found in an inducible exogenous copy of LAMP2A (Tet-off-LAMP2A mouse) where activation of the transgene in the liver of old mice restored the CMA levels close to those of young mice. Overall, comparing macroautophagy to CMA, one would say that the first one takes place early during nutritionally deprived conditions with the second one being actually activated later, when the macroautophagic activity starts to decrease [67].

**Lipofuscin and the mitochondrial-lysosomal axis theory of aging**

Accumulated evidence suggests that during the ageing process, the ability of autophagy to digest oxidatively damaged macromolecules and organelles is significantly diminished resulting in an accumulation of long-lived post-mitotic cells such as cardiomyocytes and neurons as well as lipofuscin (a non-degradable intralysosomal polymer) and aberrant proteins forming aggregates (Fig. 1) [68]. Mitochondria become defective and interaction between the defective mitochondria with lipofuscin-loaded lysosomes promotes cellular ageing process [69]. Lipofuscin, an ageing pigment, is considered a creditable marker for the ageing of cells. Lipofuscin tends to accumulate even at an early age, but rapidly progresses with the advancement of ageing process suggesting the inability of autophagy to handle the garbage disposal capacity. The mitochondrial-lysosomal axis theory of ageing [70] predicts that lysosomal dysfunction associated with ageing process is the hallmark for lipofuscin accumulation. Thus, decline of autophagy during ageing appears to be the cause for lipofuscin accumulation. In fact, both the *in vivo* and *in vitro* function of autophagy is reduced with the advancement of age. Interestingly, such reduction in age-dependent autophagy is reversed with fasting and/or CR [53, 66]. In the next section, we will discuss a number of pharmacological manipulations that can induce autophagy when it is needed.

**Signalling cascades regulating autophagy**

Before discussing pharmacological manipulation to enhance autophagy, it is necessary to understand the signalling processes for autophagy regulation (Fig. 3). Interestingly, there is striking similarities in the signalling pathways between autophagy and ageing (Fig. 4).

1. **mTOR signalling.** In yeast, there are two functionally distinct mTOR complexes, one (TORC1) is rapamycin sensitive and responds to nutrition and the other (TORC2) is rapamycin insensitive and does not respond to nutrition [71]. If mTOR signalling is inhibited, physiological characteristics of starvation appear including induction of autophagy [72]. In yeast cells, autophagy is controlled by TORC1 through autophagy-related genes (Atg) 1,
Atg13 and Atg17, all being involved in the formation of the autophagosomes and inactivation of TORC1 by rapamycin alters the phosphorylation of Atg13 thereby inducing autophagy [73]. Reduction of mTOR signalling by RNAi or knocking down of mTOR, let-363/CeTOR or the regulatory associated protein of mTOR (RAPTOR) can extend the lifespan in C. elegans [74]. Insulin/IGF-1 signalling negatively regulates autophagy through mTOR as described later [75].

2. **Insulin/IGF-1.** Similar to ageing, insulin/IGF-1 pathway is critically involved in the autophagy process. It has been known that low insulin levels can induce autophagy (such as during fasting) whereas high insulin concentration suppresses autophagy [76]. Although the precise relationship of insulin/IGF-1 between ageing and longevity is not clear, autophagy appears to be involved in the regulation of ageing through this signalling pathway. This hypothesis is supported by the observation that knockdown of ATG7 and ATG12 partially inhibits the lifespan extension of the insulin-like receptor Daf-2 mutants and causes significant shortening of the lifespan of wild-type worms [77]. Increased longevity of Daf-2 mutants is partially inhibited by the mutants of Atg6 (also known as Beclin-1), Atg-18 or Atg 8 (ubiquitin-like protein also known as LC3) [78]. These reports suggest that Insulin/IGF-1 signalling pathway is involved in the autophagy regulation of ageing. This concept receives further support from the observations that mutations of Daf-2, Age-1 (encoding the catalytic subunits of PI3K) and Pdk-1 [encoding PDK-1 (pyruvate dehydrogenase kinase)] extend lifespan [79].

3. **Ras-cAMP-PKA pathway.** This pathway plays a crucial role in the stress response and proliferation as well as longevity (Fig. 2) [80]. In a recent study, three autophagy-related proteins have been identified, Atg1, Atg13 and Atg 18, as protein kinase A (PKA) substrates in Saccharomyces cerevisiae, which act at the early stages of autophagy indicating that Ras/PKA inhibits early in the process [81]. In response to nutrient-rich conditions, two Ras GTPases, Ras1 and Ras2 activate adenylate cyclase to produce cAMP, which after binding results in the dissociation of PKA regulatory subunit Bcy1 leading to the activation of PKA catalytic subunits Tpk1, Tpk2 and Tpk3 [82]. A recent study has indicated that Ras/PKA regulates the lifespan based on the observation that type 5 adenylyl cyclase knockout mice live 30% longer compared to corresponding wild-type littermates [83].

4. **Sch9 pathway.** Sch9, the yeast PKB homologue, is a negative regulator of autophagy as its hyperactivation reduces autophagy when mTOR is inactivated by rapamycin whereas simultaneous inactivation of Sch9 and PKA without suppression of TORC1 induces autophagy, suggesting that they control autophagy in parallel (Fig. 2) [84]. It appears that longevity phenotype of PKA and Sch9 signalling are linked to each other with autophagy activation as Msn2/4 and Rim15 are involved in PKA- and Sch9-mediated regulation of autophagy [84]. PKA, Sch9 and mTOR are likely to regulate the biological processes in concert because they integrate nutrient signalling and cellular growth [85].

5. **AMPK pathway.** In response to metabolic stress, AMPK activates cellular processes to restore intracellular energy, and thus functions like an energy sensor [86]. In a recent study, AMPK was found to activate the lifespan of C. elegans and promote...
autophagy in human cells [87]. Another related study showed that myocardial ischaemia stimulated autophagy through an AMPK-dependent mechanism [88]. Ischaemic areas at risk (AAT) are capable of inducing the tumour suppressor kinase LKB1, which acting through the AMPK pathway and eukaryotic elongation factor 2F (eE2F) can activate autophagy (Fig. 3).

6. FoxO/p53 pathway. The PI3K pathway working through Akt/ PKB as well as the Sirups have been found to activate FoxO (Fig. 3). Up-regulation of FOXO induces autophagy as shown in Drosophila, C. elegans and mouse muscle fibres [89]. Crosstalking with p53, FoxO and sirtuins can regulate longevity and enhance autophagy [90].

Pharmacological manipulations to induce autophagy

There are several ways by which autophagy can be induced. The most common and well known technique is fasting. In this section, some of these techniques will be briefly discussed.

1. Fasting/CR. In most species, fasting and CR have been associated with an enhancement of lifespan by simultaneously inducing autophagy. Fasting or CR reduces plasma insulin levels and indeed exposure of Daf-2 mutant worms to CR extends lifespan, suggesting CR and insulin/IGF-1 signalling function in parallel in regulating ageing [91]. A recent study indicates that Sch9 and mTOR pathways mediate the lifespan extension of CR [92].

2. Resveratrol. Morselli et al. have shown that transgenic expression of SirT1 induces autophagy in human cells in vitro and in C. elegans in vivo [93]. Caloric restriction and resveratrol promotes longevity through the SirT1-dependent induction of autophagy [94] and improved mitochondrial function [95]. The knockdown or knockout of SirT1 prevented the induction of autophagy by resveratrol and by nutrient deprivation in human cells as well as by dietary restriction in C. elegans. Conversely, SirT1 was not required for the induction of autophagy by rapamycin or p53 inhibition, neither in human cells nor in C. elegans. The knockdown of pharmacological inhibition of SirT1 enhanced the vulnerability of human cells to metabolic stress, unless they were stimulated to undergo autophagy by treatment with rapamycin or p53 inhibition. Along the similar lines, resveratrol and dietary restriction only prolonged the lifespan of autophagy-proficient nematodes, whereas these beneficial effects of longevity were abolished by the knockdown of the essential autophagic modulator Beclin-1. This study indicates that autophagy is universally required for the lifespan-prolonging effects of caloric restriction and pharmacological SirT1 activators. Recently, the resveratrol’s ability to directly activate SirT1 and its anti-ageing effect have been questioned [96–98].

3. Spermidine. Spermidine, a precursor of spermine, is a ubiquitous polycation, which is synthesized from putrescine. These polyamines including putrescine, spermidine and spermine participate in diverse biological processes. Exogenous supply of spermidine prolongs the lifespan of several organisms including yeast, nematodes and flies simultaneously reducing age-related oxidative protein damage in mice, indicating this compound may fulfill the definition of anti-ageing drug. Spermidine induces autophagy in cultured yeast and mammalian cells, as well as in nematodes and flies and genetic inactivation of essential autophagy genes abolishes the lifespan prolonging effect of spermidine in yeast, nematodes and flies [99, 100].

4. Other chemical promoters of autophagy. Although not proven beyond any doubt, several nutriceuticals including curcumin [101], piperine [102], trehalose [103] and lithium [104] have been found to induce autophagy. Whether such autophagy is linked with lifespan extension is not yet known [103].

Future perspectives

There is overwhelming evidence that cellular mechanisms and signalling pathways regulating ageing are related to autophagy. An increase in autophagy extends life span via CR and insulin signalling. Key factors that regulate longevity and enhance autophagy such as p53, FOXOs and sirtuins, as well as protein translation factors such as elf4E and eE2F are among the putative targets in the fight against ageing. Nutriceuticals including resveratrol, spermidine, curcumin and piperine all seem to work in a complex way towards enhancement of autophagy. Future targeting of players involved in the autophagic pathway holds promise in anti-ageing or lifespan extension research.

Conflict of interest

None of the authors have any conflict of interest.

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