JGG 2017;65:110-123

Review

Extending lifespan through autophagy stimulation: a future perspective

B. Tancini, L. Urbanelli, A. Magini, A. Polchi, C. Emiliani

Department of Chemistry, Biology and Biotechnology, University of Perugia, Italy

Ageing is a natural process characterized by the gradual decline of physiological functions. In the last decades, human lifespan has considerably increased. Consequently, population ageing and the resulting increase of people affected by age-related diseases, is emerging as a major social and economic challenge in developed countries. This scenario has led to an exponential growth of research projects in the field of ageing, with the aim of identifying amenable drug targets and pharmacological interventions to extend human healthy lifespan. Extensive evidence in literature suggests that the dysfunction of autophagy, a highly conserved pathway involved in maintaining cellular homeostasis, is part of the ageing process with roles in the pathobiology of age-related diseases. Moreover, accumulating experimental data from invertebrate and vertebrate animal models demonstrate that intervening to increase lifespan also induces autophagy, suggesting that stimulating such cellular process may represent an effective strategy to increase longevity. Here, we reviewed the literature on autophagy in ageing and age-related diseases, also discussing the perspective of behavioral and pharmacological interventions that may increase healthy lifespan through autophagy stimulation.

Key words: Autophagy, Ageing, Lifespan

INTRODUCTION

Ageing, an intrinsic feature of life, is characterized by the gradual loss of capacity of organs, tissues and cells to maintain the functional and structural integrity upon perturbation by endogenous and exogenous insults. In the last decades, increased medical progresses and better living conditions have led to a dramatic lifespan increase of both developing and rich regions. Currently, ageing populations represent a global phenomenon, which is emerging as one of the major socioeconomic burden of the last century. As stated by the current European Commission Ageing Report [1], the demographic trends projected over the long term reveal that Europe is 'turning increasingly grey' in the coming decades. The projections show large and sustained increases in lifespan. In the EU, life expectancy at birth for males is expected to increase by 7.1 years over the long period, reaching 84.8 in 2060. For females, it is projected to increase by 6.0 years, reaching 89.1 in 2060. However, since the ageing process involves functional decline and increased risk of chronic diseases, the increase of life expectancy cannot straightforwardly translate into an equivalent increase in healthy life expectancy [2]. Now the progressive increase in longevity, if not accompanied by a reduction of the prevalence of chronic disabling diseases, would lead to an increased demand of assistance/support for older people and economic burden on health-care systems. This scenario has led to a growing interest among the scientific community. Researchers in the field of the biology and genetics of ageing have rushed on such a cogent topic in order to develop safe interventions to further slowdown the ageing process increasing healthy lifespan [3 4].

From a molecular point of view, ageing is an absolute example of complexity characterized by the accumulation of cellular damage promoting disease mechanisms and ultimately death. Results from studies on molecular mechanisms of ageing and age-related diseases evidenced that the age-associated cellular damage largely

Received: July 1, 2016 - Accepted: October 19, 2016

Correspondence: Brunella Tancini, Departimento di Chimica, Biologia e Biotecnologie, Università di Perugia, via Elce di Sotto 8, 06123 Perugia, Italy - Tel. +39 075 5857442 - E-mail: brunella.tancini@unipg.it

results from the alteration of only a few evolutionarily conserved genetic and biochemical pathways. In particular, the main causes impaired cellular homeostasis associated to the ageing process are: genomic instability [5], changes in nutrient sensing [6-8], mitochondrial dysfunction [9], loss of proteostasis and autophagy efficiency [10 11]. It is noteworthy that the molecular mechanisms underlying ageing-associated defects are interconnected and affect the same cellular processes responsible for most of the age-related diseases such as cancer, cardiovascular and neurodegenerative disorders [3]. Therefore, efforts aimed at identifying pharmacological interventions to slow down the molecular progress of ageing may also contribute to delay or prevent many chronic diseases. In this context, there is a general consensus that dietary, behavior and pharmacological interventions which modulate intracellular signaling pathways involved in response to nutrient availability, oxidative stress and the overall cellular protection may delay ageing and improve healthy lifespan. In particular, recent findings indicate that conserved signaling pathways, such as the insulin/insulin-like growth factor 1 (IGF1) [12], mammalian target of rapamycin complex 1 (mTORC1) [13], and the AMP-dependent protein kinase (AMPK) pathway [14] converge on autophagy to extend lifespan. On this basis, modulation of such pathway may be of great relevance to slow down ageing and delay or prevent age-related diseases though autophagy induction.

Here we focus on the role of autophagy on ageing and age-related disorders and reviewed the literature on anti-ageing interventions affecting this pathway.

AUTOPHAGY

Autophagy is an evolutionarily conserved process with an essential role in the maintenance of cellular and tissue homeostasis. The primary function of autophagy consists in degrading long-lived proteins, damaged or excess organelles or portions of cytoplasm for recycling. Autophagy contributes to cellular homeostasis also by its activation under stress conditions, such as nutrient and growth factor deprivation, oxidative damage, hypoxia or anoxia, ER stress, invasion of pathogens [9]. Moreover, as discussed in more detail below, data from experimental studies in invertebrates and vertebrates have consistently shown a tight link between autophagy and longevity [15-17]. Noteworthy, the efficiency of the autophagy pathway has been extensively reported to decrease with age and age-related diseases [11 16 18].

In mammals, three main types of autophagy have been described based on the mechanism and type of cargo delivered to the lysosome: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). Macroautophagy consists of a non-selective sequestration of cytoplasmic damaged organelles and proteins, followed by their vesicular transport to lysosomes. The process involves the formation, maturation, trafficking and subsequent degradation of double-membrane structures known as autophagosomes [19]. Although macroautophagy is generally considered to be a non-selective process, accumulating evidence has clearly shown that it can also specifically target damaged or superfluous organelles. Depending on the cargo, different forms of selective autophagy have been described including mitophagy (mitochondria), pexophagy (peroxisomes), ER-phagy (ER), xenophagy (pathogens), and others [20-22]. In microautophagy, cytosolic material is internalized for degradation in single-membrane vesicles that form through invaginations in the surface of lysosomes or late endosomes. In mammalian cells, this process has been recently shown to take place at the surface of the late endosomes (endosomal microautophagy) and to utilize the machinery required for the biogenesis of multivesicular bodies [23]. In CMA, targeted proteins are translocated across the lysosomal membrane as a complex with chaperone proteins recognized by the lysosomal-associated membrane protein 2A (LAMP-2A) receptor, which induces their unfolding and degradation. CMA selectively degrades soluble proteins containing the specific amino acid sequence KFERQ. This peptide is recognized by cytosolic chaperone proteins belonging to the HSC-70 family [24]. The balance between these three processes is fundamental for the autophagy regulation of cellular homeostasis.

THE MACROAUTOPHAGY MACHINERY

Macroautophagy (hereafter called autophagy) is the most prevalent form of autophagy. The autophagy process proceeds through (i) cargo recognition and assembly of an isolation membranes called phagophores; (ii) cargo sequestration into double membrane vesicles called autophagosomes/amphisomes; (iii) autophagosome/amphisome-lysosome fusion leading to the formation of autolysosome, and iv) cargo degradation by lysosomal hydrolytic enzymes (Fig. 1). To date, more than 35 autophagy-related genes (Atg) that are essential for autophagosome biogenesis have been identified in yeast [25]. Many of these genes have orthologs in higher eukaryotes.

Nucleation, elongation and closure

The autophagic process begins with the assembly of an isolation membrane, the phagophore. This structure is formed by the recruitment of lipids and proteins from different pre-formed organelles such as the endoplasmic

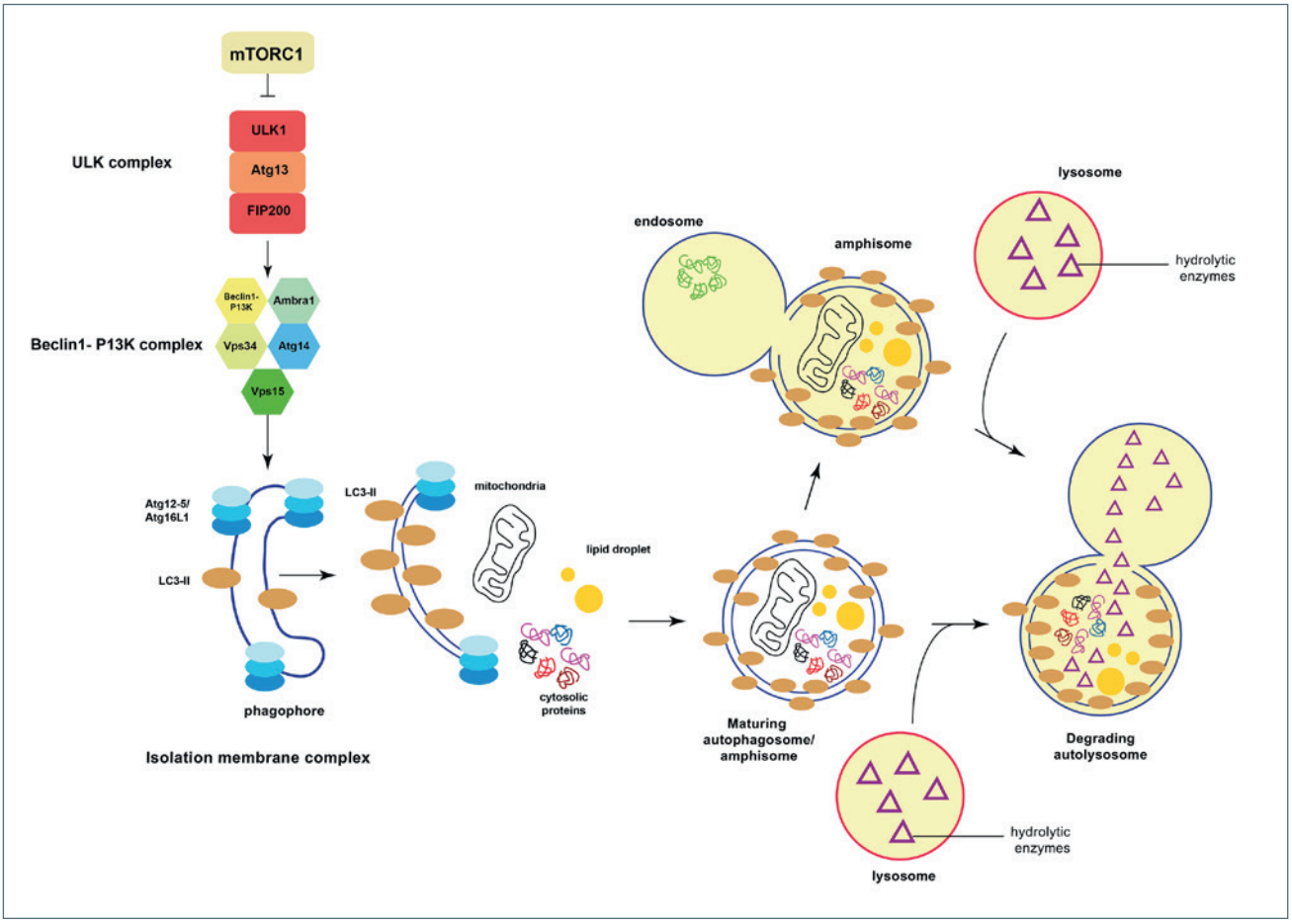

Figure 1. The macroautophagy pathway.
The autophagic process consists of a non-selective sequestration of damaged organelles, proteins and lipids, which are delivered to lysosomes for degradation and recycling. Autophagy pathway starts with the activation of the ULK complex, which in turn activates the Beclin1-PI3K complex giving rise to the recruitment of ATG proteins. The process proceeds through the assembly of an isolation membranes called phagophore and the recognition of cargo. Phagophore undergoes multiple elongation events that culminate with cargo sequestration, originating double membrane vesicles called autophagosome/amphisome. The latter fuses with the lysosome, leading to the formation of the autolysosome, where cargo degradation by lysosomal hydrolytic enzymes occurs.

reticulum, the Golgi apparatus, mitochondria or the plasma membrane [26-29]. In mammals, the process initiates through the activation of the macromolecular "ULK1/Atg13/FIP200 complex" (ULK complex) via phosphorylation of the ULK1 protein [13]. ULK complex in turn phosphorylates and activates Beclin1 and Class III phosphatidylinositol 3-kinase Vps34 (Beclin1-PI3K) complex. Beclin1, the mammalian ortholog of Atg6, is essential for the isolation membrane nucleation and phagophore assembly. Additional regulatory proteins such as UVRAG, Atg14L and AMBRA1 associates with Beclin1 and Vps34 to form a core complex which binds to the cytoskeleton. The ULK-dependent phosphorylation of AMBRA1 releases the Beclin1-PI3K complex and promotes its catalytic activity and the increase of PI3P level, which is essential for phagophore elongation and recruitment of other Atg proteins [30]. The phagophore expands to engulf intra-cellular cargo and undergoes maturation and fusion events which culminate with the formation of the autophagosome, a closed double-membrane structure. Limiting membrane elongation and closure involves two macromolecular complexes, the Atg5-Atg12 conjugate and the lipidated form of LC3 (LC3-II), whose formation is mediated by the coordinated action of two ubiquitin-like conjugation systems. The first system is represented by activated Atg7, a protein-protein ubiquitin-like ligase, which forms the Atg5-Atg12 conjugate. The second system is represented by the Atg5-Atg12 conjugate which in turn binds to Atg16L1 to form the protein-lipid ubiquitin-like ligase "Atg5-Atg12-Atg16L1 complex" [31]. This latter complex promotes the conjugation of the cytosolic LC3-I, which is

derived from the C-terminal cleavage of LC3 by Atg4, to phosphatidylethanolamine to form the autophagosome membrane-bound lipidated LC3-I, i.e. LC3-II [32]. LC3-II promotes the elongation and closure of the autophagosomal membrane and is essential for cargo selection, because of its ability to bind to the scaffolding protein p62 [33]. After sealing of the autophagosomal membrane, the Atg12–Atg5-Atg16L1 complex is released from the newly formed autophagosome, whereas LC3-II remains bound to the autophagosome membrane until this fuses with lysosomes [34].

Maturation, fusion and degradation

Autophagosome maturation consists of its fusion with lysosome to form the "autolysosome"), where the autophagosomal content is degraded by lysosomal acid hydrolases. As the autophagosome formation occurs at random sites in the cytoplasm, the autophagosome must travel to the endocytic system and fuse with late endosomes/multivesicular bodies to generate an amphisome, which finally fuses with lysosome, or alternatively it may fuse directly with lysosomes to form an autolysosome. Microtubules and the dynein motor complex are directly implicated in autophagosomal transport and fusion with lysosome [35]. The fusion step is under the control of proteins involved in intracellular membrane trafficking such as Rab GTPases, SNAREs and membrane-tethering complexes. Among GTPases, Rab7 regulates the trafficking of cargos along microtubules and participates to the fusion step with lysosomes [36], while Rab11 has been shown to promote late endosome-autophagosome fusion [37]. SNAREs are membrane-anchored proteins localized on opposing membrane compartments that interact with each other to bring the opposing lipid bilayers together and allow their fusion to occur. While the majority of SNARE proteins are localized to endosomes and synaptic vesicles, a recent study has identified Syntaxin 17 as a SNARE specifically related to autophagy [38]. Membrane-tethers are thought to facilitate the docking and fusion process by bridging the opposing membranes and/or stimulating SNARE complex formation [39]. Within the autolysosome, the sequestered cargo is degraded and released into the cytoplasm for recycling. In addition to the autophagy machinery, proper lysosomal function is also essential for efficient fusion events and cargo degradation, as underlined by the evidence that lysosomal storage disorders are characterized by autophagy failure [40].

AUTOPHAGY REGULATION

Autophagy maintains cellular homeostasis under both normal and stress conditions. Autophagy is active at basal levels in most cell types where plays a housekeeping role in maintaining the integrity of intracellular organelles and proteins. Moreover, autophagy is induced during energy or nutrient deprivation in order to recycle intracellular components, restore the energy or nutrient deficiency and promote cellular survival. Consequently, cells have developed control mechanisms that tightly modulate autophagic activity in response to diverse environmental cues (Fig. 2). A major role in autophagy regulation is played by the mTORC1 [13]. mTORC1 functions to integrate a wide range of intra- and extracellular signals such as insulin, growth factors, mitogens, energy and amino acids level to control protein synthesis, metabolism and promote cellular growth [41]. The core components of mTORC1 are the serine/threonine kinase mTOR (target of rapamycin), the scaffolding subunit Raptor (regulatory associated protein of mTOR), the kinase inhibitors DEPTOR (DEP domain containing mTOR-interacting protein), PRAS40 (proline-rich Akt substrate of 40 kDa) and mLST8 (mTOR associated protein) [42]. Under basal conditions, the mTORC1 complex associates with the ULK1/Atg13/FIP200 complex via a direct interaction between Raptor and ULK1 and inhibits autophagy through the phosphorylation and inactivation of ULK1 and Atg13 [43]. When cellular energy is depleted by nutrient deprivation, the mTORC1-dependent phosphorylation sites in ULK1 are rapidly dephosphorylated. Then, ULK1 phosphorylates itself, Atg13 and FIP200 leading to the assembly of the ULK1/Atg13/FIP200 complex, and to the induction of autophagy [44]. Alternatively, ULK1 is phosphorylated and activated by AMPK [14], a kinase activated during nutrient deprivation and low energy charge. Activated AMPK induces autophagy by phosphorylating ULK1 at residues distinct from those phosphorylated by mTOR [45]. Therefore, the coordinated phosphorylation of ULK1 by mTORC1 and AMPK possibly controls autophagic flux in response to metabolic requirements. Furthermore, AMPK also activates autophagy by directly inhibiting mTORC1 through phosphorylation and activation of tuberous sclerosis complex 2 (TSC2) and Raptor [46 47].

Besides, recent studies demonstrated that the transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, enhances autophagy by positively controlling the expression of lysosomal and Atg genes [48]. TFEB activity and nuclear translocation depend on its phosphorylation status. Under basal conditions, mTORC1 phosphorylates TFEB which is retained into cytosol. Cellular conditions such as stress, starvation and low energy inhibit mTORC1and induce TFEB dephosphorylation and nuclear translocation. In the nucleus, TFEB activates the transcription of its target genes leading to lysosomal biogenesis and autophagy pathway activation [49].

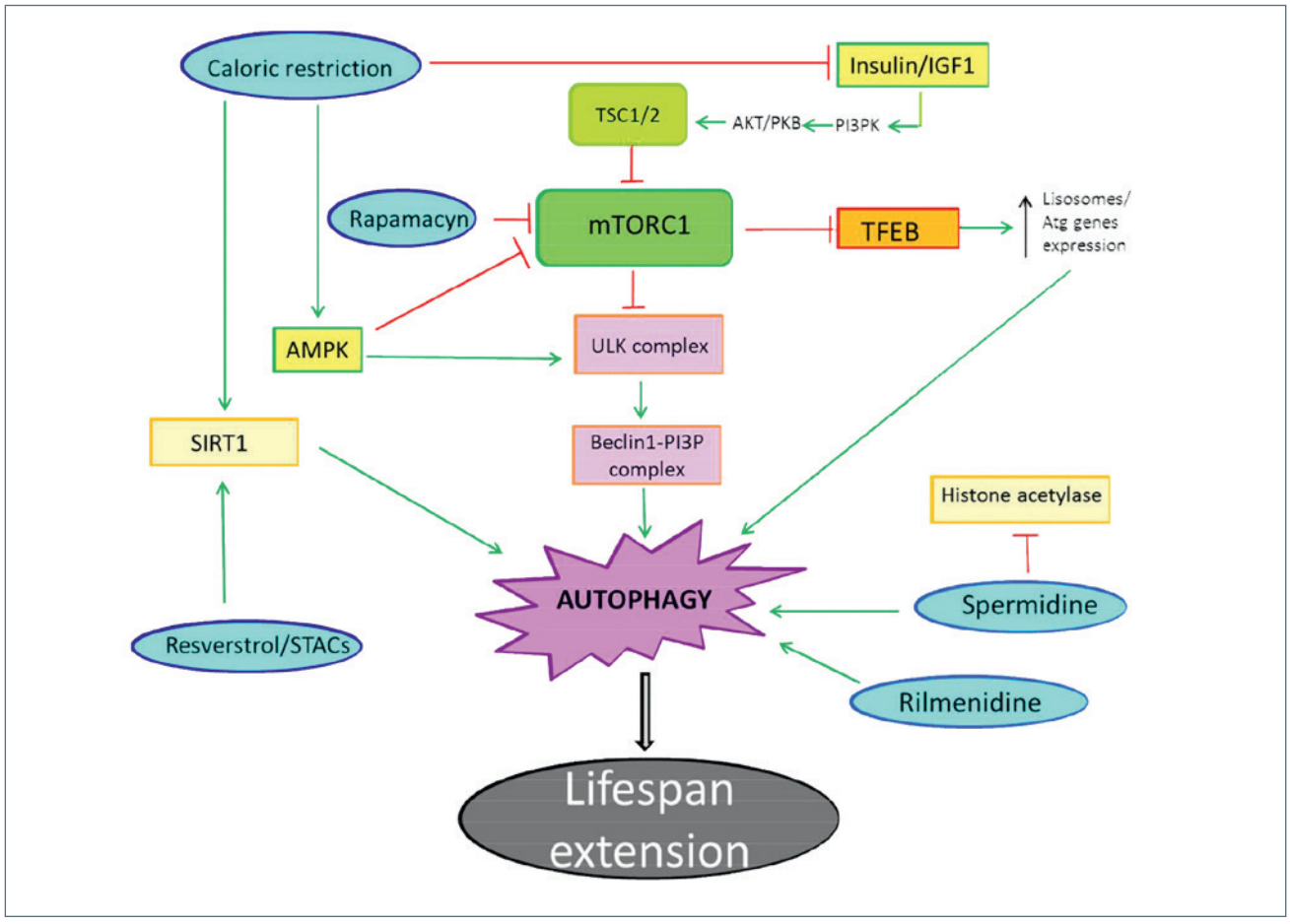

Figure 2. Signaling pathways and stress responses converging on autophagy to regulate lifespan.
Insulin/IGF-1 and TORC1 pathways inhibition, or SIRT1 and AMPK pathways activation increase lifespan through autophagy induction in a wide variety of species. Under basal conditions mTORC1 inhibits autophagy by associating with the ULK1/Atg13/FIP200 complex and inhibiting it. Under nutrient deprivation or low energy, different signaling pathways inactivate TOR kinase activity, thus inducing autophagy through the release and activation of the ULK1/Atg13/FIP200 complex. The interventions targeting different pathways which contribute to aging regulation by autophagy stimulation and result in improved health and enhanced lifespan, are shown. Green arrows: activating inputs; red bars: inhibitory interactions; light blue boxes: anti-aging interventions stimulating autophagy through activation/suppression of different signaling pathways which regulate longevity.

Accumulating findings indicate that different signaling pathways and environmental factors may converge on both mTOR and autophagy to regulate the lifespan of many species [50 51]. For instance, the insulin/IGF1 hormonal system, which activates mTORC1 through the insulin receptor/phosphoinositide 3-kinase/AKT signaling pathway [52], has been shown to accelerate ageing and increase mortality in many organisms [53]. According to this, evidences have been reported that deletion of AKT/PKB prolongs life in *Saccharomyces cerevisiae* (*S. cerevisiae*), *Caenorhabditis elegans* (*C. elegans*), and *Drosophila melanogaster* (*D. melanogaster*) [53] while inactivation of mTOR, which is upregulated by the PI3K/AKT/PKB cascade, extends lifespan in yeast, flies, worms and mice [54-57]. Activation of autophagy by caloric restriction, which increases healthy lifespan in many organism including humans, is mediated by the inhibition of the insulin/IGF-1 signaling pathway leading to mTOR activity inhibition [53].

Epigenetic factors may also affect ageing through autophagy modulation. Manipulation of enzymes regulating the acetylation status of chromatin (sirtuins, histone acetyltransferases, histone deacetylases) has been reported to influence lifespan in many organism models [58 59]. In particular, recent data demonstrate that the Sirtuin-type chromatin remodeling factors implicated in ageing regulation may require autophagy for their lifespan extension effect in both invertebrates and vertebrates [60]. Activated SIRT1, a NAD^+-dependent protein deacetylases which overexpression has an anti-ageing

effect in mice [61], induces autophagy by transcriptional activation of some autophagy genes through deacetylation of chromatin proteins at several autophagy-related loci [62]. Analogously, inhibition of histone acetylases strongly induces autophagy [63]. These evidences indicate that protein acetylation at chromatin level may play a general role in the regulation of the autophagy pathway.

ROLE OF AUTOPHAGY IN AGEING AND AGE-RELATED DISEASES

As mentioned above, numerous evidences have been reported indicating that autophagy efficiency decreases with age in almost all organisms. Besides, the progressive decrease of autophagy activity is considered one of the causes of the functional decline of biological systems during ageing supporting a link between autophagy and ageing process [11 18]. Early findings suggesting a relationship between autophagy and lifespan come from studies on model organisms. For instance, decreased lifespan has been observed in short-lived yeast mutants with defective autophagy [64] and studies on the nematode *C. elegans* and the fruit fly *D. melanogaster* also revealed a decreased lifespan in mutants with defective Atg genes function [65 66]. Moreover, it has been reported that mice models with selectively deleted essential Atg genes display an "ageing-like" phenotype and diseases that are associated with ageing, indicating the need of functional autophagy to sustain lifespan and healthspan also in mammals [67-69]. On the other hand, genetic or pharmacological intervention strategies that extend lifespan in model systems are often associated with autophagy stimulation [57 65 70].

Although numerous studies have established a tight connection between autophagy and ageing, the relationship between autophagy and lifespan at molecular level is not yet completely understood. The primary role of autophagy is the degradation and recycling of intracellular components to ensure cellular clearance and maintenance of homeostasis. The gradual loss of autophagy efficiency with ageing results in the intracellular accumulation of damaged substances and organelles that severely compromises the cellular function leading to a decreased ability of the cells, and ultimately of the organism, to survive [71]. Biochemical measurements of protein degradation rate in rat liver revealed an age-dependent decline in autophagy activity that correlate with a decreased lysosomal-dependent breakdown and progressive accumulation of damaged proteins [71]. Therefore, the link between autophagy and ageing seems to be closely associated to the progressive accumulation of damaged macromolecules and organelles, a common feature of the ageing process. Such types of damage include increased misfolded or aggregated macromolecules [72], defective mitochondria [73], lipid droplet accumulation [17], lipofuscin-loaded lysosomes accumulation [74]. Moreover, it has been demonstrated that the reduced efficiency of autophagy pathway may be due, at least in part, to impaired ability of the lipofuscin-loaded lysosomes to fuse with autophagosomes [74 75]. In addition, accumulated aberrant cellular components can actively compromise cellular function by interfering with protein turnover and proper signaling pathways [76].

Cellular damage is continuously generated by both exogenous and endogenous cues. For example, mitochondrial respiration produces reactive free radical derivatives such as reactive oxygen species (ROS), which are able to oxidize many biological molecules. Therefore, the oxidative damage is a cumulative phenomenon during the entire life of an organism and autophagy plays a protective role by removing damaged macromolecules and organelles. On this regard, accumulating findings in yeast and mammalian cells suggest the existence of an intriguing crosstalk between ROS production and autophagy regulation [77-79] and numerous studies indicate that several molecular players are involved in the complex interplay between ROS and autophagy. For instance, under starvation, ROS regulates autophagy through the activation of the conserved regulatory protein AMPK, a positive regulator of autophagy [80]. Oxidative stress can also activate the tumor suppressor protein p53 that is able to trigger autophagy through its transcriptional activity [81]. Conversely, it has been reported that the cytoplasmic form of p53 inhibits autophagy through the p53 inducible protein TIGAR [82]. ROS also activate redox-sensitive proteases that are involved in autophagy. In particular, the essential autophagy protein ATG4 is a Cys-dependent protease directly targeted by mitochondrial ROS under nutrient deprivation. The redox-dependent inactivation of ATG4 leads to increased autophagosome formation by inhibition of LC3 de-lipidation [83]. Although the precise link between ROS and autophagy is not completely understood, collectively it seems conceivable that ROS induce autophagy to reduce oxidative damage. It is well known that the ageing process is also accompanied by increasing oxidative stress [84]. So it appears that oxidative stress may contribute to ageing not only directly but also indirectly, by increasing the request of autophagic degradation of damaged material.

In conclusion, the decline in autophagy function with ageing results in the accumulation of damaged substances and organelles leading to cellular dysfunction and, ultimately, death. On the other hand, numerous genetic studies reveal that the overexpression of autophagy essential genes promotes lifespan extension

and improves health span in multiple model organisms confirming the tight connection between autophagy, lifespan and ageing [85 87].

The loss of autophagy efficiency with ageing is an important factor contributing to several age-related disorders including neurodegeneration, cancer, infection, cardiovascular dysfunction and muscle atrophy [3 88]. For example, many studies support that autophagy failure contributes to many late-onset human neurodegenerative diseases. One of the common pathological features of these pathologies is the accumulation of cytosolic aggregate-prone proteins, such as mutant tau, α-synuclein and mutant huntingtin which are distinctive features of Alzheimer, Parkinson and Huntington diseases, respectively [89 90]. The autophagy impairment reduces cellular clearance of the soluble forms of aggregate-prone proteins, leading to increasing aggregation and cell toxicity [67 91]. Importantly, it has been demonstrated that autophagy induction prevents the age-dependent accumulation of damage in neurons and reduces toxicity in several organism models [93-95]. Although many evidence supports a connection between autophagy and different types of cancer, the exact role of autophagy in tumorigenesis is still controversial [96]. A strong support indicating that autophagy may act as a tumor-suppressor pathway comes from the observations that essential autophagic genes are mutated in human cancers suggesting that impairment of autophagic machinery might contribute to tumorigenesis [97-99]. As autophagy protects cells from the insults caused by the accumulation of unfolded, dysfunctional, aggregated proteins, increased ROS level and DNA damage, the cellular damages derived from autophagic deficit may favor cancer occurrence in certain cells [100]. On this regard, several findings suggest that a number of autophagy genes also have properties of onco-suppressors [101-103] and loss of Beclin1 increases tumor cell proliferation [99]. On the other hand, the pro-survival function of autophagy could allow the mutant cells to survive in conditions of hypoxia and nutrient deprivation, as those occurring in the core of solid tumors. Indeed, it has been found that malignant cells maintain autophagic activity, albeit at low levels, to promote tumor survival under metabolic stress conditions [104 105]. Besides, it has been reported that autophagy is necessary for the maintenance of the energetic requirements of cancer cells [106 107]. In conclusion, functional autophagy appears to be essential in preventing tumorigenesis, but its pro-survival role under stressing condition may support malignant cells to allow tumor growth and development. Therefore, autophagy upregulation may have beneficial effects as prophylactic treatment, while reducing autophagy may be of benefit in existing tumors.

Autophagy is also an important defense mechanism against infections. By sequestering and degrading intracellular-invading bacterial pathogens, autophagic degradation takes part to the innate immune response against a number of microbial pathogens that invade eukaryotic cells [108 109]. For instance, it has been reported that Group A Streptococcus is internalized into autophagosome-like structures and degraded in an Atg5-dependent manner [110]. A number of other pathogens, like *Toxoplasma gondii*, *Listeria monocytogenes, Salmonella enterica*, or *Rickettsia conorii*, have been found to be targets of autophagic degradation [108 111]. Therefore, failure of autophagy pathway may represent an advantage for bacterial pathogens, leading to an increased susceptibility to infectious agents in the elderly.

AUTOPHAGY STIMULATION PROMOTES LIFESPAN EXTENSION

The ageing process is mainly characterized by the lifelong accumulation of cellular damage. Therefore, enhancing or preserving the activity of mechanisms eliminating cellular damage may be useful to decrease not only the rate of damage accumulation, but may also slow down degenerative processes which occur with ageing. On the other hand, it is well documented that autophagy, which has a crucial role on clearing cellular damage, decrease with age in diverse organisms ranging from yeast to mammals. On this basis, restoration of normal autophagic function may represent a useful therapeutic strategy aimed at increase lifespan and preventing, or at least delaying, age-related disorders. On this regard, many studies indicate that genetic, dietary or pharmacological interventions extending lifespan in many model organisms also induce autophagy. On the contrary, numerous interventions extending lifespan require autophagy for their longevity-promoting effects (Fig. 2). Among this, the beneficial effects of caloric restriction (CR), i.e. the reduction of total calorie intake by 20-50% without malnutrition or the diminished intake of specific dietary components [112], have been extensively documented [113 114]. Data from experimental studies in invertebrates and rodents have consistently shown that reduced food intake, avoiding malnutrition, play major roles in promoting health and longevity [53 115]. In addition, numerous CR clinical studies in humans have given very encouraging results raising the possibility to consider CR for long-term clinical trials focused on healthspan [116]. For instance, evidence has been reported indicating that a CR of around 15% may be most favorable against mortality during ageing [117]. Moreover, long-term CR with adequate intake of nutrients results in several metabolic adaptations that reduce the risk of developing type 2 diabetes, hypertension,

cardiovascular disease, neurodegeneration and cancer [114]. The effect of CR on autophagy induction is mainly mediated by the inhibition of the insulin/IGF1 signaling pathway that ultimately leads to inhibition of mTOR activity and promote survival during ageing. In *S. cerevisiae*, starvation causes down-regulation of the TOR-S6K and Ras-adenylate cyclase-PKA pathways, and induces the activation of stress resistance transcription factors regulating many protective and metabolic genes [118]. Analogously, in yeast, flies, worms, and mammals, fasting reduces circulating IGF-1 and leads to down-regulation of PI3K-AKT, mTOR and PKA pathways and the activation of multiple transcription factors related to stress resistance and survival [53 119]. The anti-ageing effects of CR have been functionally linked to autophagy also through the activation of both the energy sensors Sirtuin1 (SIRT1) and AMPK [45 73 120]. In particular, it has been reported that SIRT1, which is essential for lifespan extension mediated by CR [121], is also a potent inducer of autophagy [122 123]. Analogously, AMPK activation may extend lifespan in worms and mice [124 125] and may also induce autophagy [45 126]. These observations indicate that the lifespan extension mediated by CR is related to autophagy activation and suggest a strong correlation between CR and autophagy.

Apart from CR, several pharmacological interventions with both clinically approved compounds and natural compounds have been reported to delay ageing through activation of the autophagic machinery in diverse species from yeasts, flies, nematodes up to mice [127]. Interestingly, autophagy is often activated in association with mTOR pathway inhibition and inhibitors of this pathway are widely used as inducers of autophagy [128]. Rapamycin, a direct inhibitor of the mTOR kinase [129], has already been approved for uses as anticancer agent, antifungal antibiotic and immunosuppressant [130-133]. Rapamycin treatment has been shown to extend lifespan as well as healthspan in different animal models of ageing [134]. Importantly, it has been demonstrated that the lifespan-extending effect of rapamycin is strictly dependent on autophagy induction in yeast, nematodes and flies [135 136]. In addition to enhancing longevity, it has been reported that rapamycin treatments clear aggregate-prone proteins in cell and animal models of many age-related neurodegenerative diseases such as Alzheimer, Parkinson and Huntington diseases through autophagy induction [137-140]. Although inhibition of mTOR activity clearly has beneficial effects during ageing, long-term administration of rapamycin and rapamacyn derivatives required in chronic disorders may have a range of undesirable side effects including increased infections, reduced male fertility, hyperlipidemia, insulin resistance, and diabetes mellitus [141-144]. To date, the effectiveness of rapamacyn and rapalogs to slow ageing in humans remains to be determined, but the results obtained so far clearly indicate that it is possible to slow down ageing and delay the onset or progression of age-related diseases laying the basis for encouraging future developments.

Other convincing evidence that induction of autophagy leads to extension of lifespan come from different studies using natural compounds such as spermidine and resveratrol. Spermidine, which is a naturally occurring ubiquitous polyamine, is among the most effective inducers of autophagy [145 146]. In yeast, spermidine acts as an inhibitor of histone acetylases and affects the transcription of several genes, some of which are involved in the autophagic degradation machinery [63]. It has been reported that exogenous supply of spermidine promotes longevity via induction of autophagy in yeast, flies and worms [63 148]. Likewise, nutrient supplementation with food containing spermidine increases longevity and reduced age-related pathology in mice [149]. On the other hand, the inhibition of autophagy by genetic manipulation abolishes the beneficial effects of spermidine on lifespan in both flies and worms [63], indicating a strong correlation between the autophagy induction and the pro-survival effect mediated by spermidine. Because of spermidine proved to be non-toxic in mice and human studies [150], it should be considered as potential intervention strategy promoting healthspan on human.

Resveratrol, a polyphenol found in grape berry skin, red wine and other plants, has been reported to have positive effects on lifespan in a range of organisms including mice [151 152]. It is an anti-oxidant natural compound with anti-inflammatory, anti-cancer and antiviral properties [153 154]. Resveratrol is also a potent inducer of autophagy [123 146]. Findings have been reported indicating that the promoting lifespan effect of resveratrol requires autophagy and inhibition of autophagy pathway by genetic or pharmacological manipulation abolishes these beneficial effects [123 146]. The autophagy stimulation induced by resveratrol is mediated by direct activation of SIRT1, a member of sirtuin deacetylases [146 156]. Based on the observation that resveratrol activates SIRT1, which is a deacetylase [157-159], and spermidine inhibits acetylases [63], it has been suggested that these two compounds mediate longevity through convergent pathways. A proteomic study on human colon carcinoma cells revealed that they induce convergent acetylproteome modifications that control the autophagic network [160]. Another polyphenol compound with antioxidant and anti-inflammatory activities similar to those of resveratrol is oligonol. A recent study demonstrated that oligonol, analogously to resveratrol, may act as an anti-ageing molecule by inducing autophagy via up-regulation of SIRT1 gene expression and the AMPK pathway [161].

This finding further supports that Sirtuins may represent attractive drug targets to promote healthy ageing. The discovery of natural sirtuin-activating compounds (STACs) with beneficial effects on healthspan prompted the production of more potent and bioavailable synthetic SIRT1 activators [151]. These compounds, as well as resveratrol, mimic the beneficial effects of CR and have also shown promising results in treating age-related diseases such as cancer, type 2 diabetes, inflammation, cardiovascular disease and neurodegeneration in different animal models [151 162]. Resveratrol and synthetic STACS have also been extensively tested in humans with conflicting reports of their efficacies [163-165]. Interestingly, a recent study demonstrated a non-linear dose response for the protective effects of resveratrol in humans and mice [166]. This finding may be useful to clarify the contradictory results so far obtained with STACs in human contributing to understand the actual efficacy of these compounds as anti-ageing agent in human.

In the last years, efforts to identify safer autophagy promoting drugs have led to the identification of new small molecules able to activate autophagy without interfere with the mTOR pathway [167].

Among these, rilmenidine, a well-tolerated United States Food and Drug Administration-approved anti-hypertensive drug, has been reported to induce autophagy in mice and in primary neuronal culture and also attenuate Huntington's disease symptoms in a mouse model of the disease [168]. Therefore, rilmenidine may be considered for the treatment of Huntington's disease and related disorders that commonly occur with ageing.

CONCLUSIONS

Ageing is a natural multifactorial process characterized by the gradual accumulation of cellular damage culminating in impaired function, increased susceptibility to develop diseases and ultimately death. Accumulating evidence indicates that ageing can be delayed in animal models by genetic and small molecule interventions, raising the possibility that anti-ageing therapies are an effective opportunity also in humans. Autophagy, an evolutionarily conserved process, promotes the elimination of dysfunctional organelles, protein aggregates and intracellular pathogens in order to maintain cellular homeostasis in both normal and stress conditions. Many findings have clearly established the molecular and mechanistic relationship existing between autophagy and ageing. On this regard, studies carried out in different organisms indicated that interventions aimed at increasing lifespan and healthspan also stimulate autophagy and *vice versa*. For instance, enhanced longevity can be achieved by CR, mTORC1 complex inhibition and sirtuin-activating compounds. All of these interventions act through autophagy stimulation. Therefore, autophagy represents a potential target to slow down the ageing process and prevent/delay age-related diseases. However, most of the compounds described so far as effective anti-ageing agents target multiple longevity signaling pathways upstream of autophagy and their long-term administration may have undesired side effects, such as alteration of cell growth and homeostasis. Further studies should focus on the precise nature of regulatory interactions between longevity pathways and the autophagy machinery, in order to develop safer and more specific anti-ageing drugs. One limitation of testing new compounds with anti-ageing effects in mammals is the lack of suitable in vivo models to explore long-term effects of drugs on the ageing process. Although mice are the main mammalian model in ageing studies, they are not suited for large-scale unbiased screening of new drugs due to macroscopic differences with humans in terms of metabolic and gene regulation frames. Alternatively, since most of the pathways modulating the ageing process in mammals are highly conserved and have homologies with short-living organisms, yeast, flies, and worms, these organisms have been extensively used as pilot experimental models to test candidate anti-ageing drugs, the identification of which may accelerate the discovery of treatments that extend the lifespan/healthspan in other species, potentially including humans. The results obtained so far on intervention strategies aimed at stimulate autophagy have laid the basis for encouraging future developments. The ultimate goal is not only to increase longevity, but also to prevent or delay pathogenic mechanisms of age-related diseases.

ACKNOWLEDGEMENTS

This research project was supported by Fondazione Cassa di Risparmio di Perugia Grant 2016.0050.021 and by Fondi per la Ricerca di Base 2014-University of Perugia-Dept. of Chemistry,Biology and Biotechnology.

References

1 *The 2015 Ageing Report Economic and budgetary projections for the 28 EU Member States* (http://ec.europa.eu/economy_finance/publications/).

2 Hung WW, Ross JS, Boockvar KS, et al. *Association of chronic diseases and impairments with disability in older adults: a decade of change?* Med Care 2012;50:501-7.

3 Longo VD, Antebi A, Bartke A, et al. *Interventions to slow aging in humans: are we ready?* Aging Cell 2015;14:497-510.

[4] Kennedy BK, Berger SL, Brunet A, et al. *Geroscience: linking aging to chronic disease*. Cell 2014;159:709-13.

[5] Moskalev AA, Shaposhnikov MV, Plyusnina EN, et al. *The role of DNA damage and repair in aging through the prism of Koch-like criteria.* Ageing Res Rev 2013;12:661-84.

[6] López-Otín C, Blasco MA, Partridge L, et al. *The hallmarks of aging*. Cell 2013;153:1194-217.

[7] Inoki K, Kim J, Guan KL. *AMPK and mTOR in cellular energy homeostasis and drug targets.* Annu Rev Pharmacol Toxicol 2012;52:381-400.

[8] Houtkooper RH, Pirinen E, Auwerx J. *Sirtuins as regulators of metabolism and healthspan.* Nat Rev Mol Cell Biol 2012;13:225-38.

[9] Lionaki E, Markaki M, Palikaras K, et al. *Mitochondria, autophagy and age-associated neurodegenerative diseases: new insights into a complex interplay*. Biochim Biophys Acta 2015;1847:1412-23.

[10] Powers ET, Morimoto RI, Dillin A, et al. *Biological and chemical approaches to diseases of proteostasis deficiency*. Annu Rev Biochem 2009;78:959-91.

[11] Martinez-Lopez N, Athonvarangkul D, Singh R. *Autophagy and aging*. Adv Exp Med Biol 2015;847:73-87.

[12] Oldham S, Hafen E. *Insulin/IGF and target of rapamycin signaling: a TOR de force in growth control.* Trends Cell Biol 2003;13:79-85.

[13] Hosokawa N, Hara T, Kaizuka T, et al. *Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy.* Mol Biol Cell 2009;20:1981-91.

[14] Kim E, Goraksha-Hicks P, Leufeld TP, et al. *Regulation of TORC1 by Rag GTPases in nutrient response*. Nat Cell Biol 2008;10:935-45.

[15] Jia K, Levine B. *Autophagy and longevity: lessons from C. elegans.* Adv Exp Med Biol 2010;694:47-60.

[16] Komatsu M, Waguri S, Chiba T, et al. *Loss of autophagy in the central nervous system causes neurodegeneration in mice.* Nature 2006;441:880-4.

[17] Singh R, Kaushik S, Wang Y, et al. *Autophagy regulates lipid metabolism*. Nature 2009;458:1131-5.

[18] Cuervo AM. *Autophagy and aging: keeping that old broom working*. Trends Genet 2008;24:604-12.

[19] Yang Z, Klionsky DJ. *Mammalian autophagy: core molecular machinery and signaling regulation.* Curr Opin Cell Biol 2010;22:124-31.

[20] Bellu AR, Kiel JA. *Selective degradation of peroxisomes in yeasts.* Microsc Res Tech 2003;61:161-70.

[21] Bernales S1, McDonald KL, Walter P. *Autophagy counterbalances endoplasmic reticulum expansion during the unfolded protein response.* PLoS Biol 2006;4:e423.

[22] Castrejón-Jiménez NS, Leyva-Paredes K, Hernández-González JC, et al. *The role of autophagy in bacterial infections.* Biosci Trend 2015;9:149-59.

[23] Sahu R, Kaushik S, Clement CC, et al. *Microautophagy of cytosolic proteins by late endosomes.* Dev Cell 2011;20:131-9.

[24] Arias E, Cuervo AM. *Chaperone-mediated autophagy in protein quality control.* Curr Opin Cell Biol 2011;23:184-9.

[25] Araki Y, Ku WC, Akioka M, et al. *Atg38 is required for autophagy specific phosphatidylinositol 3-kinase complex integrity.* J Cell Biol 2013;203:299-313.

[26] Hamasaki M, Furuta N, Matsuda A, et al. *Autophagosomes form at ER-mitochondria contact sites.* Nature 2013;495:389-93.

[27] Ge L, Melville D, Zhang M, et al. *The ER-Golgi intermediate compartment is a key membrane source for the LC3 lipidation step of autophagosome biogenesis.* Elife 2013;2:e00947.

[28] Hailey DW, Rambold AS, Satpute-Krishnan P, et al. *Mitochondria supply membranes for autophagosome biogenesis during starvation.* Cell 2010;141:656-67.

[29] Cuervo AM. *The plasma membrane brings autophagosomes to life*. Nat Cell Biol 2010;12:735-7.

[30] Axe EL, Walker SA, Manifava M, et al. *Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum.* J Cell Biol 2008;182:685-701.

[31] Romanov J, Walczak M, Ibiricu I, et al. *Mechanism and functions of membrane binding by the Atg5-Atg12/Atg16 complex during autophagosome formation.* EMBO J 2012;31:4304-17.

[32] Walczak M, Martens S. *Dissecting the role of the Atg12-Atg5-Atg16 complex during autophagosome formation.* Autophagy 2013;9 424-5.

[33] Ichimura Y, Kumanomidou T, Sou YS, et al. *Structural basis for sorting mechanism of p62 in selective autophagy*. J Biol Chem 2008;283:22847-57.

[34] Weidberg H, Shvets E, Shpilka T, et al. *LC3 and GATE-16/GABARAP subfamilies are both essential yet act differently in autophagosome biogenesis.* EMBO J 2010;29:1792-802.

[35] Jahreiss L, Menzies FM, Rubinsztein DC. *The itinerary of autophagosomes: from peripheral formation to kiss-and-run fusion with lysosomes.* Traffic 2008;9:574-87.

[36] Huotari J, Helenius A. *Endosome maturation.* EMBO J 2011;30:3481-500.

[37] Fader CM, Sanchez D, Furlan M, et al. *Induction of autophagy promotes fusion of multivesicular bodies with autophagic vacuoles in k562 cells.* Traffic 2008;9:230-50.

[38] Itakura E, Kishi-Itakura C, Mizushima N. *The hairpin-type tail anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes*. Cell 2012;151:1256-69.

[39] Brocker C, Engelbrecht-Vandre S, Ungermann C. *Multisubunit tethering complexes and their role in membrane fusion*. Curr Biol 2010;20:R943-R52.

[40] Lieberman AP, Puertollano R, Raben N, et al. *Autophagy in lysosomal storage disorders*. Autophagy 2012;8:719-30.

[41] Jewell JL, Guan KL. *Nutrient signaling to mTOR and cell growth*. Trends Biochem Sci 2013;38:233-42.

[42] Laplante M, Sabatini DM. *mTOR signaling in growth control and disease*. Cell 2012;149:274-93.

[43] Ganley IG, Lam du H, Wang J, et al. *ULK1.ATG13.FIP200*

complex mediates mTOR signaling and is essential for autophagy. J Biol Chem 2009;284:12297-305.

44 Kamada Y, Yoshino K, Kondo C, et al. *Tor directly controls the Atg1 kinase complex to regulate autophagy*. Mol Cell Biol 2010;30:1049-58.

45 Egan DF, Shackelford DB, Mihaylova MM, et al. *Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy.* Science 2011;331:456-61.

46 Inoki K, Zhu T, Guan KL. *TSC2 mediates cellular energy response to control cell growth and survival.* Cell 2003;115:577-90.

47 Gwinn DM, Shackelford DB, Egan DF, et al. *AMPK phosphorylation of raptor mediates a metabolic checkpoint*. Mol Cell 2008;30:214-26.

48 Settembre C, Di Malta C, Polito VA, et al. *TFEB links autophagy to lysosomal biogenesis.* Science 2011;332:1429-33.

49 Settembre C, Zoncu R, Medina DL, et al. *A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB*. EMBO J.2012;31:1095-108.

50 Rabinowitz JD, White E. *Autophagy and metabolism*. Science 2010;330:1344-8.

51 Zoncu R, Efeyan A, Sabatini DM. *mTOR: from growth signal integration to cancer, diabetes and ageing.* Nat Rev Mol Cell Biol 2011;12:21-35.

52 Yoshida S, Hong S, Suzuki T, et al. *Redox regulates mammalian target of rapamycin complex 1 (mTORC1) activity by modulating the TSC1/TSC2-Rheb GTPase pathway*. J Biol Chem 2011;286:32651-60.

53 Fontana L, Partridge L, Longo VD. *Extending healthy life span – from yeast to humans*. Science 2010;328:321-6.

54 Kaeberlein M, Powers RW, Steffen KK, et al. *Regulation of yeast replicative life span by TOR and Sch9 in response to nutrients.* Science 2005;310:1193-6.

55 Kapahi P, Zid BM, Harper T, et al. *Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway*. Curr Biol 2004;14:885-90.

56 Vellai T, Takacs-Vellai K, Zhang Y, et al. *Genetics: influence of TOR kinase on lifespan in C. elegans*. Nature 2003;426:620.

57 Harrison DE, Strong R, Sharp ZD, et al. *Rapamycin fed late in life extends lifespan in genetically heterogeneous mice.* Nature 2009;460:392-5.

58 Longo VD. Linking sirtuins. *IGF-I signaling, and starvation*. Exp Gerontol 2009;44:70-4.

59 Pengelly AR, Copur O, Jackle H, et al. *A histone mutant reproduces the phenotype caused by loss of histone-modifying factor Polycomb.* Science 2013;339:698-9.

60 Salminen A, Kaarniranta K. *SIRT1: regulation of longevity via autophagy*. Cell Signal 2009;21:1356-60.

61 Satoh A, Brace CS, Rensing N, et al. *Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH.* Cell Metab 2013;18:416-30.

62 Lee IH, Cao L, Mostoslavsky R, et al. *A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy.* Proc Natl Acad Sci USA 2008;105:3374-9.

63 Eisenberg T, Knauer H, Schauer A, et al. *Induction of autophagy by spermidine promotes longevity.* Nat Cell Biol 2009;11:1305-14.

64 Matecic M, Smith DL, Pan X, et al. *A micro-array-based genetic screen for yeast chronological aging factors.* PLoS Genet 2010;6:e1000921.

65 Toth ML, Sigmond T, Borsos E, et al. *Longevity pathways converge on autophagy genes to regulate life span in Caenorhabditis elegans*. Autophagy 2008;4:330-8.

66 Simonsen A, Cumming RC, Brech A, et al. *Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult Drosophila*. Autophagy 2008;4:176-84.

67 Hara T, Nakamura K, Matsui M, et al. *Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice.* Nature 2006;441:885-9.

68 Komatsu M, Waguri S, Ueno T, et al. *Impairment of starvation-induced and constitutive autophagy in Atg7- deficient mice.* J Cell Biol 2005;169:425-34.

69 Cadwell K, Liu JY, Brown SL, et al. *A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells*. Nature 2008;456:259-63.

70 Bjedov I, Toivonen JM, Kerr F, et al. *Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster.* Cell Metabolism 2010;11:35-46.

71 Vellai T. *Autophagy genes and ageing*. Cell Death Differ 2009;16:94-102.

72 Ward W. *Protein degradation in the aging organism.* Prog Mol Subcell Biol 2002;29:35-42.

73 Kim I, Rodriguez-Enriquez S, Lemasters JJ. *Selective degradation of mitochondria by mitophagy.* Arch Biochem Biophys 2007;462:245-53.

74 Brunk UT, Terman A. *Lipofuscin: mechanisms of age related accumulation and influence on cell function.* Free Radic Biol Med 2002;33:611-9.

75 Brunk UT, Terman A. *Lipofuscin: mechanisms of age-related accumulation and influence on cell function.* Free Radic Biol Med 2002;33:611-9.

76 Szweda PA, Camouse M, Lundberg KC, et al. *Aging, lipofuscin formation, and free radical-mediated inhibition of cellular proteolytic systems.* Ageing Res Rev 2003;2:383-405.

77 Azad MB, Chen Y, Gibson SB. *Regulation of autophagy by reactive oxygen species (ROS): implications for cancer progression and treatmen*t. Antioxid Redox Signal 2009;11:777-90.

78 Chen Y, Azad MB, Gibson SB. *Superoxide is the major reactive oxygen species regulating autophagy.* Cell Death Differ 2009;16:1040-52.

79 Zhang J, Kim J, Alexander A, et al. *A tuberous sclerosis complex signaling node at the peroxisome regulates mTORC1 and autophagy in response to ROS*. Nat Cell Biol 2013;15:1186-96.

80 Li L, Chen Y, Gibson SB. *Starvation-induced autophagy is*

regulated by mitochondrial reactive oxygen species leading to AMPK activation. Cell Signal 2013;25:50-65.

[81] Vessoni AT, Filippi-Chiela EC, Menck CF, et al. *Autophagy and genomic integrity*. Cell Death Diffe 2013;20:1444-54.

[82] Bensaad K, Cheung EC, Vousden KH. *Modulation of intracellular ROS levels by TIGAR controls autophagy.* EMBO J 2009;28:3015-26.

[83] Scherz-Shouval R, Shvets E, Fass E, et al. *Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4*. EMBO Journal 2007;26:749-1760.

[84] Sohal RS. *Role of oxidative stress and protein oxidation in the aging process*. Free Radic Biol Med 2002;33:37-44.

[85] Pyo JO, Yoo SM, Ahn HH, et al*. Overexpression of Atg5 in mice activates autophagy and extends lifespan.* Nat Commun 2013;4:2300.

[86] Yang L, Li P, Fu S, et al. *Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance.* Cell Metab 2010;11:467-78.

[87] Simonsen A, Birkeland HC, Gillooly DJ, et al. *Alfy, a novel FYVE domain-containing protein associated with protein granules and autophagic membranes*. J Cell Sci 2004;117:4239-51.

[88] Mizushima N, Levine B, Cuervo AM, et al. *Autophagy fights disease through cellular self-digestion.* Nature 2008;451:1069-75.

[89] Levine B, Kroemer G. *Autophagy in the pathogenesis of disease*. Cell 2008;132:27-42.

[90] Bjorkoy G, Lamark T, Brech A, et al. *p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death*. J Cell Biol 2005;171:603-14.

[91] Komatsu M, Waguri S, Chiba T, et al. *Loss of autophagy in the central nervous system causes neurodegeneration in mice*. Nature 2006;441:880-4.

[92] Hara T, Nakamura K, Matsui M, et al. *Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice.* Nature 2006;441:885-9.

[93] Simonsen A, Cumming RC, Brech A, et al. *Promoting basal levels of autophagy in the nervous system enhances longevity and oxidant resistance in adult Drosophila*. Autophagy 2008;4:176-84.

[94] Ravikumar B, Vacher C, Berger Z, et al. *Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease*. Nat Genet 2004;36:585-95.

[95] Berger Z, Ravikumar B, Menzies FM, et al. *Rapamycin alleviates toxicity of different aggregate-prone proteins*. Hum Mol Genet 2006;15:433-42.

[96] Liu J, Debnath J. *The evolving, multifaceted roles of autophagy in cancer*. Adv Cancer Res 2016;130:1-53.

[97] Coppola D, Khalil F, Eschrich SA, et al. *Down-regulation of Bax-interacting factor-1 in colorectal adenocarcinoma.* Cancer 2008;113:2665-70.

[98] Liang C, Feng P, Ku B, et al. *Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG.* Nat Cell Biol 2006;8:688-99.

[99] Yue Z, Jin S, Yang C, et al. *Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor*. Proc Natl Acad Sci USA 2003;100:15077- 82.

[100] Jin S. *Autophagy, mitochondrial quality control, and oncogenesis.* Autophagy 2006;2:80-4.

[101] Marino G, Salvador-Montoliu N, Fueyo A, et al. *Tissue-specific autophagy alterations and increased tumorigenesis in mice deficient in Atg4C/autophagin-3*. J Biol Chem 2007;282:18573-83.

[102] Qu X, Yu J, Bhagat G, et al. *Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene.* J Clin Invest 2003;112:1809-20.

[103] Takahashi Y, Coppola D, Matsushita N, et al. *Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis*. Nat Cell Biol 2007;9:1142-51.

[104] Wang RC, Levine B. *Autophagy in cellular growth control*. FEBS Lett 2010;584:1417-26.

[105] Sato K, Tsuchihara K, Fujii S, et al. *Autophagy is activated in colorectal cancer cells and contributes to the tolerance to nutrient deprivation.* Cancer Res 2010;67:9677-84.

[106] Lock R, Roy S, Kenific CM, et al. *Autophagy facilitates glycolysis during ras mediated oncogenic transformation.* Mol Biol Cell 2010;22:165-78.

[107] Lock R, Kenific CM, Leidal AM, et al. *Autophagy-dependent production of secreted factors facilitates oncogenic RAS-driven invasion.* Cancer Discov 2014;4:466-79.

[108] Levine B. *Eating oneself and uninvited guests: autophagy-related pathways in cellular defense.* Cell 2005;120:159-62.

[109] Schmid D, Munz C. *Innate and adaptive immunity through autophagy*. Immunity 2007;27:11-21 .

[110] Nakagawa I, Amano A, Mizushima N, et al. *Autophagy defends cells against invading group A Streptococcus.* Science 2004;306:1037-40.

[111] Ogawa M, Nakagawa I, Yoshikawa Y, et al. *Streptococcus-, Shigella-, and Listeria-induced autophagy.* Methods Enzymol 2009;452:363-81.

[112] Masoro EJ. *Overview of caloric restriction and ageing*. Mech Ageing Dev 2005;126:913-22.

[113] Omodei D, Fontana L. *Calorie restriction and prevention of age-associated chronic disease*. FEBS Lett 2011;585:1537-42.

[114] Colman RJ, Anderson RM, Johnson SC, et al. *Caloric restriction delays disease onset and mortality in rhesus monkeys.* Science 2009;325:201-4.

[115] Fontana L, Partridge L. *Promoting health and longevity through diet: from model organisms to humans.* Cell 2015;16:106-18.

[116] Longo VD, Mattson MP. *Fasting: molecular mechanisms and clinical applications*. Cell Metab 2014;19:181-92.

[117] Willcox BJ, Willcox DC. *Caloric restriction, caloric restriction mimetics, and healthy aging in Okinawa: controversies and clinical implications.* Curr Opin Clin Nutr Metab Care 2014;17:51-8.

[118] Wei M, Fabrizio P, Hu J, et al. *Life span extension by calorie*

restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. PLoS Genet 2008;4:e13.

[119] Cheng CW, Adams GB, Perin L, et al. *Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression*. Cell Stem Cell 2014;14:810-23.

[120] Cantó C, Jian LQ, Deshmukh AS, et al. *Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle.* Cell Metab 2010;11:213-9.

[121] Chen D, Steele AD, Lindquist S, et al. *Increase in activity during calorie restriction requires Sirt1.* Science 2005;310:1641.

[122] Lee IH, Cao L, Mostoslavsky R, et al. *A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy.* Proc Natl Acad Sci USA 2008;105:3374-9.

[123] Morselli E, Maiuri MC, Markak, M, et al. *Caloric restriction and resveratrol promote longevity through the sirtuin-1-dependent induction of autophagy*. Cell Death Dis 2010;1:e10.

[124] Mair W, Morantte I, Rodrigues AP, et al. *Lifespan extension induced by AMPK and calcineurin is mediated by CRTC-1 and CREB.* Nature 2011;470:404-8.

[125] Onken B, Driscoll M. *Metformin induces a dietary restriction-like state and the oxidative stress response to extend C. elegans Healthspan via AMPK, LKB1, and SKN-1.* PLoS One 2010;5:e8758.

[126] Liang J, Shao SH, Xu ZX, et al. *The energy sensing LKB1-AMPK pathway regulates p27(kip1) phosphorylation mediating the decision to enter autophagy or apoptosis*. Nat Cell Biol 2007;9:218-24.

[127] Gelino S, Hansen M. *Autophagy-an emerging anti-aging mechanism*. J Clin Exp Pathol 2012;(Suppl 4).pii:006.

[128] Rubinsztein DC, Marino G, Kroemer G. *Autophagy and aging.* Cell 2011;148:682-95.

[129] Sarbassov DD, Ali SM, Sabatini DM. *Growing roles for the mTOR pathway*. Curr Opin Cell Biol 2005;17:596-603.

[130] Eng CP, Sehgal SN, Vezina C. *Activity of rapamycin (AY-22,989) against transplanted tumors*. J Antiobiot (Tokyo) 1984;37:1231-7.

[131] Vezina C, Kudelski A, Sehgal SN. *Rapamycin (AY 22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle*. J Antibiot (Tokyo) 1975;28:721-6.

[132] Martel RR, Klicius J, Galet S. *Inhibition of the immune response by rapamycin, a new antifungal antibiotic.* Can J Physiol Pharmacol 1977;55:48-51.

[133] Ingle GR, Sievers TM, Holt CD. *Sirolimus: continuing the evolution of transplant immunosuppression.* Ann Pharmacother 2000;34:1044-55.

[134] Kennedy BK, Pennypacker JK. *Drugs that modulate aging: the promising yet difficult path ahead.* Transl Res 2014;163:456-65.

[135] Bjedov I, Toivonen JM, Kerr F, et al. *Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster.* Cell Metab 2010;11:35-46.

[136] Alvers AL, Wood MS, Hu D, et al. *Autophagy is required for extension of yeast chronological life span by rapamycin*. Autophagy 2009;5:847-9.

[137] Spilman P, Podlutskaya N, Hart MJ, et al. *Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease*. PLoS One 2010;5:e9979.

[138] Majumder S, Richardson A, Strong R, et al. *Inducing autophagy by rapamycin before, but not after, the formation of plaques and tangles ameliorates cognitive deficits.* PLoS One 2011;6:e25416.

[139] Webb JL, Ravikumar B, Atkins J, et al. *Alpha-Synuclein is degraded by both autophagy and the proteasome.* J Biol Chem 2003;278:25009-13.

[140] Berger Z, Ravikumar B, Menzies FM, et al. *Rapamycin alleviates toxicity of different aggregate-prone proteins.* Hum Mol Genet 2006;15:433-42.

[141] Zuber J, Anglicheau D, Elie C, et al. *Sirolimus may reduce fertility in male renal transplant recipients*. Am J Transplant 2008;8:1471-9.

[142] Lamming DW, Ye L, Katajisto P, et al. *Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity.* Science 2012;335:1638-43.

[143] Gyurus E, Kaposztas Z, Kahan BD. *Sirolimus therapy predisposes to new-onset diabetes mellitus after renal transplantation: a longterm analysis of various treatment regimens*. Transplant Proc 2011;43:1583-92.

[144] McCormack FX, Inoue Y, Moss J, et al. *Efficacy and safety of sirolimus in lymphangioleiomyomatosis.* N Engl J Med 2011;364:1595-606.

[145] Sigrist SJ, Carmona-Gutierrez D, Gupta VK, et al. *Spermidine-triggered autophagy ameliorates memory during aging*. Autophagy 2014;10:178-9.

[146] Morselli E, Galluzzi L, Kepp O, et al. *Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol.* Aging (Albany) 2009;1:961-70.

[147] Eisenberg T, Knauer H, Schauer A, et al. *Induction of autophagy by spermidine promotes longevity.* Nat Cell Biol 2009;11:1305-14.

[148] Minois N, Carmona-Gutierrez D, Bauer MA, et al. *Spermidine promotes stress resistance in Drosophila melanogaster through autophagy-dependent and -independent pathways*. Cell Death Dis 2012;3:e401.

[149] Soda K, Dobashi Y, Kano Y, et al. *Polyamine-rich food decreases age-associated pathology and mortality in aged mice.* Exp Gerontol 2009;44:727-32.

[150] Soda K, Kano Y, Sakuragi M, et al. *Long-term oral polyamine intake increases blood polyamine concentrations*. J Nutr Sci Vitaminol (Tokyo) 2009;55:361-6.

[151] Hubbard BP, Sinclair DA. *Small molecule SIRT1 activators for the treatment of aging and age-related diseases.* Trends Pharmacol Sc 2014;35:146-54.

[152] Pearson KJ, Baur JA, Lewis KN, et al. *Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span*. Cell Metab 2008;8:157-68.

[153] Zhang F, Liu J, Shi JS. *Anti-inflammatory activities of resveratrol in the brain: role of resveratrol in microglial activation.* Eur J Pharmacol 2010;636:1-7.

[154] Jang M, Cai L, Udeani GO, et al. *Cancer chemopreventive activity of resveratrol, a natural product derived from grapes.* Science 1997;275:218-20.

[155] Yiu CY, Chen SY, Chang LK, et al. *Inhibitory effects of resveratrol on the Epstein-Barr virus lytic cycle.* Molecules 2010;15:7115-24.

[156] Borra MT, Smith BC, Denu J. *Mechanism of human SIRT1 activation by resveratrol*. J Bio Chem 2005;280:17187-95.

[157] Baur JA, Sinclair DA. *Therapeutic potential of resveratrol: the in vivo evidence*. Nat Rev Drug Discov 2006;5:493-506.

[158] Lagouge M, Argmann C, Gerhart-Hines Z, et al. *Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1alpha*. Cell 2006;127:1109-22.

[159] Dai H, Kustigian L, Carney D, et al. *SIRT1 activation by small molecules: kinetic and biophysical evidence for direct interaction of enzyme and activator*. J Biol Chem 2010;285:32695-703.

[160] Morselli E, Marino G, Bennetzen MV, et al. *Spermidine and resveratrol induce autophagy by distinct pathways converging on the acetylproteome*. J Cell Biol 2011;192:615-29.

[161] Seul-Ki P, Rak-Kyun, Ji-Ae K, et al. *Oligonol promotes anti-aging pathways via modulation of SIRT1-AMPK-Autophagy Pathway*. Nutr Res Pract 2016;10:3-10.

[162] Graff J, Kahn M, Samiei A. *A dietary regimen of caloric restriction or pharmacological activation of SIRT1 to delay the onset of neurodegeneration*. J. Neurosci 2013;33:8951-60.

[163] Venkatasubramanian S, Noh RM, Daga S, et al. *Cardiovascular effects of a novel SIRT1 activator, SRT2104, in otherwise healthy cigarette smokers*. J Am Heart Assoc 2013;2:e000042.

[164] Timmers S, Konings E, Bilet L, et al. *Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans*. Cell Metab 2011;14:612-22.

[165] Yoshino J, Conte C, Fontana L, et al. *Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance*. Cell Metab 2012;16:658-64.

[166] Cai H, Scott E, Kholghi A, et al. *Cancer chemoprevention: evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice*. Sci Transl Med 2015;7:298ra117.

[167] Sarkar S, Perlstein EO, Imarisio S, et al. *Small molecules enhance autophagy and reduce toxicity in Huntington's disease models.* Nature Chemical Biology 2007;3:331-8.

[168] Rose C, Menzies FM, Renna M, et al. *Rilmenidine attenuates toxicity of polyglutamine expansions in a mouse model of Huntington's disease*. Hum Mol Genet 2010;19:2144-53.