Solicited opinion/review to Nature Ageing

Autophagy in healthy ageing and disease

Option 1: Manipulating autophagy to promote healthy ageing

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Abstract
Autophagy eliminates subcellular components via lysosome mediated degradation, a fundamental cellular process essential for homeostasis, development, differentiation and survival. While autophagy is intimately linked to health, the intricate communication between autophagy, ageing and disease, remains unclear. The cargos of autophagy include nucleic acids, proteins, lipids, organelles, and pathogens. The broad spectrum of substrates associated with autophagy raises a key question: how do distinct autophagic mechanisms influence tissue and organismal homeostasis in the long-term? This review examines several emerging features of autophagy and postulates how they may be linked to ageing and disease development and progression. We also discuss the pre-clinical evidence to use modulators of autophagy for the suppression of age-related pathologies, in particular neurodegenerative diseases. Finally, we highlight key questions and novel research avenues that will facilitate studies aimed at revealing new links between autophagy and the hallmarks of ageing. We describe the diverse ways in which autophagy influences cellular health and how its gradual decline orchestrates the different manifestations of ageing and disease. Understanding the precise interplay between functionally active autophagy and the risk for age-related pathologies will elucidate the ageing landscape across organisms and eventually facilitate the development of clinical applications that promote healthy ageing.

Keywords: autophagy; ageing; neurodegeneration; Alzheimer's disease; mitophagy; autophageing; NAD^+; Rapamycin; Spermidine; cGAS-STING

1. Ageing and autophagy

Ageing is a biological process that is characterised by a time-dependent cellular and functional decline, resulting in reduced quality of life for the organism[1]. Consistently, ageing is a primary driving force of age-related pathologies, such as cardiovascular, neoplastic and neurodegenerative diseases, including stroke, several ataxias, Alzheimer's disease/AD and other types of dementia among others. Collectively, age-related ailments result in a formidable global socio-economic burden and a significant healthcare challenge[2,3]. Therefore, identifying therapeutic interventions that promote "healthy ageing" and simultaneously halt the progression of multiple age-related pathological conditions is of paramount importance[2].

Among the many molecular changes associated with old age, altered autophagy has emerged as a consistent feature of ageing across species. However, recent advances in understanding the diversity of autophagy cargos, and the temporal and spatial effects of impaired autophagy regulation on tissue homeostasis, have revealed the complex and multi-factorial relationship between autophagy and ageing. Here we examine the relationships between autophagy, ageing and disease, and propose novel links between specific autophagic processes and long-term tissue health, and the implications for anti-ageing therapeutics.

2. Compromised autophagy is a hallmark of ageing

Research over the last decade has revealed that the process of autophagy can take many different forms. Autophagy (from the Greek words "*auto*", meaning "*self*," and "*phagein*", meaning "*to eat*") is a highly conserved eukaryotic pathway for lysosomal degradation and recycling of cellular components such as defective organelles and aggregates of misfolded proteins[4]. The process of autophagy was first described in the 1960s, but it was the identification of autophagy-related genes (*ATG*) in the 1990s that propelled the major breakthroughs in unveiling the mechanistic complexities of autophagy[5-12]. There are three major types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) (**Fig. 1a-c**), all of which involve delivery of substrates to the lysosome for degradation (detailed reviews[13,14]). Macroautophagy (hereafter referred to as autophagy) was originally thought of as an unselective, bulk degradation process. However, the discovery of selective autophagy receptors with p62/SQSTM1 being the first changed this notion[15,16]. Today autophagy is recognised as a highly selective cellular clearance mechanism that is associated with the maintenance of cellular homeostasis, as well as the cytoplasmic quality control process[17,18]. Based on the specificity of cargos, selective autophagy can be further classified into many sub-types, such as elimination of mitochondria by mitophagy (**Fig. 1d**), endoplasmic reticulum (ER) by ER-phagy (**Fig. 1e**), part of the nucleus by nucleophagy (**Fig. 1f**), lysosomes by lysophagy (**Fig. 1g**), and pathogens by xenophagy (**Fig. 1h**), among others. Linkages of these selective autophagy pathways to ageing, longevity, and diseases will be discussed in details below. The core process of macroautophagy has been described in detail elsewhere. However, in brief, the core process of autophagy begins after inhibition of the mechanistic target of rapamycin (mTOR) or activation of 5' AMP-activated protein kinase (AMPK). This is followed by membrane nucleation and phagophore formation, phagophore elongation and phagophore maturation, before the autophagosome fuses with the lysosome for cargo degradation and recycling. Key proteins involved in each steps are presented (**Fig. 1 i**).

A growing body of evidence underscores that the process of autophagy is reduced during ageing in diverse organisms[1]. Studies in *Caenorhabditis elegans*, rodents and human cells have demonstrated an age-dependent reduction in lysosomal proteolytic function, thereby impairing autophagic flux[19-22], exacerbating cellular dysfunction and contributing to age-related diseases[1,23,24]. Further evidence stemming from *Drosophila* has revealed that age is associated with the reduced expression of several *Atg* genes (*Atg2*, *Atg8a*, blue cheese gene / *Bchs*), which are pivotal for both autophagy induction and execution[25]. In aged WT mice, autophagy is reduced in the neurons of the brain as evidenced by reduced rate of autophagolysosomal fusion and decreased lysosomal accumulation of autophagy substrates

in aged hypothalamus[26]. Convergent findings in humans suggested that the expression of autophagy-related genes, such as *ATG5*, *ATG7* and *BECN1*, declines with age[27]. Moreover, the development and progression of several human pathologies are highly associated with age-dependent autophagy deficits[28-30]. Collectively these studies demonstrate that gradual decline in proteins of the autophagic machinery, and reduced delivery of cargo to lysosomes, occurs with age, thereby, implicating compromised autophagy as a cardinal feature of the ageing organism.

Autophagy is highlighted as a central denominator of the ageing process, but is it a mediator of youth and longevity? Indeed, both unselective and selective autophagy are implicated in lifespan extension in a range of experimental models. Transcriptomic profiling in yeast (*Saccharomyces cerevisiae*) provided evidence for defective autophagy amongst short-lived compared to long-lived mutants[31]. In addition, selective mutation(s) and/or knockdown of genes encoding proteins of the autophagic machinery in *C. elegans* (*lgg-1*, *unc-51*, *bec-1*, *atg-7*, *atg-12* and *atg-18*), *Drosophila* (*Atg3* and *Atg8a*) and mice (*Atg5*, *Atg7*, and *Becn1*) shorten lifespan and healthspan[1,14,25,32]. Consistent with these observations, the systemic genetic knockout of the autophagy components (*Becn1*, *Atg5*, *Atg9,* and *Atg13*) are lethal in mice, thereby highlighting the importance of autophagy in development[33]. In addition, knockdown of genes encoding transcription factors that regulate autophagy, such as transcription factor EB (*TFEB;* orthologue in *C. elegans hlh-30*) and Forkhead Box O (*FOXO*; orthologue in *C. elegans daf-16*) shortened lifespan in both wild-type (WT) and long-lived *daf-2* mutants[34].

Studies in long-lived mutant animals, in particular, the extended lifespan of *C. elegans daf-2* (insulin/IGF-1 receptor) loss-of-function mutant animals, is dependent on autophagic flux. Indeed, selective mutation/knockdown of *bec-1*, *lgg-1*, *atg-7* or *atg-12* abolish lifespan extension of DAF-2 deficient nematodes[1,14]. Formation of long-lived dauer worms, a larval hibernation stage, is also associated with increased autophagy and is dependent on autophagy genes *atg-1*, *atg-7*, *atg-8/lgg-1* and *atg-18,* underlining the essential role of autophagy in organismal adaptation during challenging conditions [35].

Genetic or pharmacological upregulation of autophagy extends lifespan in animals. Promotion of autophagy by overexpression of *Atgs* in *Drosophila* (*Atg1* and *Atg8a*) and mice (*Atg5*) extended lifespan[25,36,37]. Similarly, BCL2 mutations that disrupt the BECN1-BCL2 complex increases basal autophagic flux, leading to lifespan extension and improved healthspan extension in both male and female mice[38]. Additionally, overexpression of regulators of the autophagy machinery in *C. elegans* and *Drosophila*, such as AMPK enhanced autophagy in diverse tissues, which in turn extended lifespan[14,37], and upregulation of *hlh-30* enhanced autophagy and extended lifespan in *C. elegans*[39]. Rapamycin, an inhibitor of the mTOR pathway, fed late in life extended median and maximal lifespan of both female and male mice[40].

Accumulating evidence in aged mice as well as pathological rodent models that recapitulate characteristic features of human diseases have been shown to exhibit compromised autophagy as an underlying mechanism. In particular, age-associated dysregulation of autophagy (demonstrated by the accumulation of autophagosomes), possibly due to impaired lysosomal fusion and/or degradation, is associated with cellular dysfunction and/or death, which contributes to neurodegeneration as well as cardiac and skeletal muscle ageing[41-45]. In hematopoietic stem cells (HSC) autophagy has been shown to delay ageing via activation of downstream sirtuins-3 (SIRT3), a key mitochondrial protein capable of rejuvenating blood and protects against oxidative stress in mice and human HSC-enriched cells[46].

Moreover, autophagy has been indicated to be a critical mechanism of immune memory in mice and levels of the endogenous autophagy-inducing metabolite spermidine fall in human T cells with age. In fact, spermidine supplementation in T cells for old donors restores autophagy levels to that observed in young donors via the translation factor eIF5A and transcription factor TFEB[47]. Furthermore, spermidine administration in a mouse model of mild cognitive

impairment (MCI), a transitional phase between AD and healthy ageing, displayed an improvement in degradation of misfolded proteins accompanied by delayed age-related memory decline; thereby implicating autophagy as a mechanism of action[48]. Additional evidence stemming from genetic upregulation of p62, demonstrated that increased autophagy facilitated the degradation of AD-associated β-amyloid (Aβ) and protected against neurodegeneration and memory loss in the APP/PS1 mouse model of AD[49].

Whilst the dysregulation of autophagy underlies ageing and/or disease phenotypes; excessive autophagy also contributes to deteriorating cellular function in ageing. In particular, recent evidence demonstrates that an age-dependent decline in Rubicon, a negative regulator of autophagy, in adipocytes, exacerbates metabolic disorders[50]. Similarly, suppressing autophagy exclusively in the intestines of post-reproductive adults has been proposed to prevent the emergence of age-related pathologies in *C. elegans*[51]. These observations suggestthat the maintenance of functional autophagy is essential for healthy cellular and organismal ageing, and that dysregulation of autophagy in either direction, whether insufficient or excessive, contributes to cellular dysfunction and functional decline of an organism.

A summary of autophagy-related genes linked to longevity and disease is provided in **Tables 1** and **2**. Furthermore, several interventions known to promote lifespan, including dietary restriction and treatment with pharmacological agents, such as rapamycin, spermidine and NAD^+ precursors among others, require intact autophagic machinery. In totality, these findings reinforce the notion that autophagy stimulation is required and sufficient to maintain organismal homeostasis and extent longevity in multiple model organisms (discussed in detail below)[1]. An overview of autophagy inducers linked to enhanced longevity and improved health is presented in **Table 3**.

Together, these findings support that: (i) autophagy is compromised during the process of ageing; (ii) aggravation of autophagy shortens lifespan in various experimental animal models; and (iii) promotion/restoration of autophagy contributes to lifespan extension as well as healthspan improvement of diverse organisms. These observations highlight autophagy as a key regulator of ageing. However, they do not address an important fundamental question: How does autophagy facilitate long-term cell and tissue health?

3. The many faces of autophagy in health and ageing

3.1. Autophagy and protein homeostasis

Protein homeostasis (proteostasis) collapse is a central hallmark of ageing and disease that is characterized by the appearance of misfolded, mislocalised and aggregated proteins. While the age-related loss of proteostasis is documented in numerous tissues, age-dependent protein aggregation is strongly linked to neurodegenerative pathologies, such as AD, Parkinson's disease, Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS)[52,53].

Along with molecular chaperones and the ubiquitin proteasome system (UPS), autophagy is a central regulator of cellular proteostasis, either through, the degradation of soluble misfolded or oligomeric proteins via Chaperone Mediated Autophagy (CMA), the selective degradation of ubiquitin positive protein aggregates by chaperone-assisted selective autophagy or the removal of bulk protein aggregates by–macroautophagy[13,30,54]. Consistent with this, genetic perturbation of core machinery components, or regulators, of the autophagy machinery, accelerates age-related protein aggregation, shortens lifespan and promotes disease in worms, flies and mice. Conversely, increasing autophagy, genetically or pharmacologically, suppresses protein aggregation and promotes longevity[40] (and reviewed by [55]).

In *C. elegans,* loss of function mutations in *ATG6/bec-1* or *WIPI2/atg-18,* or RNAi against *bec-1, atg-9* or *lgg-1/ATG8,* increases susceptibility to protein aggregation, accelerates the onset of age-related paralysis and shortens lifespan[32,56]. Similarly, mutations in the core autophagy

components, *atg8* or *atg7*, in *Drosophila,* increase the levels of insoluble protein aggregates and reduced lifespan[25,57]. Finally, knockout of *Atg5* or *Atg7* in mouse neurons leads to the appearance of cytoplasmic inclusion bodies in the brain and early onset neurodegeneration[58-60], while knockout of LAMP2A (the primary receptor for CMA) in the liver, results in altered proteostasis and hepatic dysfunction with age[61].

Conversely, enhanced proteostasis and extended lifespan in *C. elegans* occurs when the lysosome-autophagy pathway-regulating transcription factor *hlh-30/TFEB,* or the selective autophagy receptor, *p62/SQSTM1,* are upregulated. Likewise, in *Drosophila,* overexpression of p62 or the autophagy activator, *FOXO*, reduces protein aggregation in various tissues and extends lifespan[39,62-65]. Pharmacological (clonidine, rilmenidine and rapamycin) and genetic (*atg5*) upregulation of autophagy in zebrafish, harbouring the rare tau variant p.A152T, ameliorated tau pathology[66]. Increased autophagy is also associated with enhanced clearance of protein aggregates in mammals, as systemic overexpression of *Atg5* or *Becn1* mutation disrupting BECN1-BCL2 binding improves proteostasis and promotes longevity in mice [36,67], and overexpression of the selective autophagy mediator BAG3 suppresses tau accumulation in neurons[68].

As a complement to the genetic modulation of autophagy, treatment of Drosophila[69] with the mTOR inhibitor rapamycin, suppresses age-related protein aggregation and extends lifespan in an autophagy-dependent manner, and in cell culture and fly models[70], suppresses toxicity associated with neurodegenerative disease-associated proteins, including mutant huntingtin, polyalanine expansion proteins and tau. Several other pharmacological autophagy-inducers, such as spermidine and nicotinamide have also been reported to protect against proteostasis collapse and proteotoxicity in various models of HD, AD, PD and ALS[55].

Autophagy has been linked to stem cell function, with the autophagy-mediated clearance of protein aggregates central to the activation of quiescent neuronal stem cells. Activating autophagy by overexpression of TFEB or rapamycin supplementation inhibited age-related protein aggregation and enhance neuronal and muscle stem cell function in aged mice [71-73]. Given the fact that stem cell exhaustion is intimately linked to age-related tissue dysfunction, these findings suggest that enhancing proteostasis specifically in stem cells may preserve many aspects of healthy tissue function during ageing. Collectively, these observations strongly support the notion that autophagy promotes healthy ageing by protecting cells against toxic misfolded and aggregated proteins.

3.2. Autophagy regulation of macromolecule availability

Another important role for autophagy is to ensure the availability of metabolites, including amino acids, lipids, carbohydrates and nucleic acids, especially during states of stress, such as nutrient starvation **(Fig. 1a)**. Under challenging conditions, autophagy promotes cellular metabolism and survival by recycling amino acids, which are generated from the degradation of cytosolic substrates, to replenish nutrients, produce energy and promote protein synthesis. The inability to properly recycle amino acids through autophagy is linked to growth and developmental defects in *Atg5* deficient mice and impaired growth during nitrogen starvation in *atg*-deficient yeast[74-76]. Autophagy can also be tailored to mediate the availability of carbohydrates, lipids and nucleic acids through three main cellular processes: glycophagy, lipophagy and RNA/DNA-phagy, respectively.

3.2.1. Glycophagy

Glucose is the primary energy source for cellular metabolism. It is stored as glycogen and metabolism of glucose is tightly regulated in a tissue-dependent manner (i.e., liver: maintain blood glucose level; muscle: source of cellular energy). However, various conditions resulting in metabolic stress, such as starvation, stimulate glycogen breakdown in order to augment cellular glucose levels and promote metabolic activity[77]. The selective clearance of glycogen via autophagy, referred to as glycophagy, plays a crucial role in glucose homeostasis. In

response to nutrient-deficiency, the energy sensor AMPK is activated, which in turn inhibits the mTOR complex 1 (mTORC1), leading to activation of the ULK1 kinase, which is important for induction of autophagy (AMPK-mTORC1-ULK1 triad)[77]. Recent findings in yeast demonstrated that Atg11 is necessary to facilitate the interaction between AMPK homolog Snf1 with ULK1 homolog Atg1 upon glucose starvation to promote autophagy[78]. The LC3-interacting region (LIR) motif, also known in yeast as Atg8 family interacting motif (AIM), in starch-binding domain-containing protein 1 (STBD1) may allow cells to physically link glycogen to GABARAPL1, facilitating the transport of glycogen to lysosomes for degradation **(Fig. 1a,** third pathway from left**)**[79]. In parallel to glycophagy, other pathways, such as β-oxidation, may maintain cellular bioenergetics to compensate for glucose deprivation[80]. Autophagy also plays a pivotal role in maintaining cell function, not only in glucose starvation, but also in conditions of excess glucose. In particular, high glucose levels were associated with mitochondrial dysfunction, generation of reactive oxygen species (ROS), and induction of autophagy in endothelial progenitor cells (EPCs)[81].

Under conditions of impaired autophagy, the accumulation of glycogen contributes to the pathogenesis of age-related diseases. Pompe disease, a lysosomal storage disorder has an impaired ability of the lysosome to degrade glycogen due to deficiency in the lysosomal hydrolytic enzyme acid α-glucosidase (GAA). This results in accumulation of lysosomal glycogen in many tissues, predominantly in skeletal and cardiac tissues, leading to progressive lethal skeletal myopathy, respiratory and cardiac defects[82]. Impaired tissue function results from an inability of the lysosome to degrade glycogen leading to energy deficiency in skeletal muscle. For infantile-onset Pompe disease[83], a promising therapeutic intervention is administration of recombinant human GAA. Furthermore, dysfunctional autophagy-mediated accumulation of glycogen has been demonstrated to be the cause of neurodegeneration in a mouse model of Lafora disease, which is suppressed when glycogen synthase is deleted[84]. These findings indicate that glycogen accumulation might be a cause, rather than a consequence, of impaired autophagy, resulting in impaired cellular function and disease. Glycophagy, therefore, is essential for cellular function and survival, suggesting that levels of glycophagy could determine organismal health, and possibly longevity.

3.2.2. Lipophagy

The intracellular storage and utilization of lipids are critical to maintain cellular energy homeostasis. In response to starvation, triglycerides stored in lipid droplets are hydrolysed by specific lipases into free fatty acids for energy metabolism. Lipid droplets can also undergo selective degradation by autophagy, termed lipophagy, as an alternative mechanism for regulating lipid homeostasis **(Fig. 1a,** fourth pathway from left**)**[85]. To date, a specific receptor coupling lipid droplets to autophagosomes, and trafficking to lysosomes, has not yet been identified, although LC3-mediated engulfment of lipid droplets has been observed[86]. Moreover, CMA has been implicated in degradation of the lipid droplet-associated proteins perilipin 2 (PLIN2) and perilipin 3 (PLIN3)[87]. The lysosomal acid lipase is involved in the degradation of lipid droplets; in particular, lipolysis is conducted primarily by adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL), and selective knockdown of ATGL and HSL in mice results in selective inhibition of lipid droplet degradation, whilst other autophagy processes (i.e., degradation of proteins and organelles) serve as a compensatory mechanism to replenish the reduced availability of energy substrates [88]. An age-dependent decline in basal autophagy in the liver may underlie the accumulation of hepatic lipids, which in turn has been proposed to contribute to metabolic conditions as well as impairing autophagy, a vicious cycle promoting ageing[85]. Indeed, age-dependent accrual of lipid droplets and ectopic fat deposition are highly interconnected with age-dependent decline of autophagy and/or autophagic defects[89,90]. Autophagy and LIPL-4-dependent lipolysis are both upregulated in germline-less *C. elegans* and work interdependently to prolong lifespan[91]. Cellular supplementation with NAD^+, which stimulates autophagy and subtypes of autophagy, including mitophagy, and stimulates the activity of the NAD^+-dependent SIRT1 and SIRT3 pathways, reduced fat accumulation and increased lifespan in high-fat fed and progeroid animals[92-94], highlighting the importance of

autophagic degradation of lipids in healthspan and lifespan. Further studies on the molecular mechanisms of lipophagy and their impact on cellular homeostasis will shed light on the relationship between autophagy, metabolism and ageing.

3.2.3. Autophagic degradation of nucleic acids: RNAs (RNautophagy / RNAphagy) and DNAs (DNautophagy / DNAphagy)

Nucleic acids can also be degraded by autophagy. RNA/DNA are targeted for lysosomal degradation via several pathways, including LC3-dependent autophagic degradation of stress granules (condensates of proteins and RNAs)[95], a p62 and NDP52-dependent autophagic degradation of retrotransposon RNA[96], a lysosomal membrane protein LAMP2C-dependent direct binding to RNA (also for DNA[97]) for lysosomal degradation[98], and a lysosomal putative RNA/DNA transporter SIDT2 (SID1 transmembrane family, member 2)-mediated direct update of RNA (and DNA[99]) for lysosomal degradation[100]. At present, little is known about whether and how RNAphagy and DNAphagy affect health and ageing. However, it is reasonable to suggest that nucleic acid turnover is essential for health, as accumulation of damaged or unnecessary DNA and RNA in the cytosol promotes inflammation, cancer, diseases and even accelerated ageing[101-103]. DNA damage triggers autophagy and subtypes of autophagy that are considered as cell survival responses[104]; however, genetic or age-dependent impairment of DNA repair leads to genomic instability, cellular dysfunction, cell death and accelerated ageing[103]. Cytoplasmic intruding of exogenous DNA (e.g., microbial DNA), or accumulation of endogenous DNA from the nucleus or mitochondria activates the cyclic GMP-AMP (cGAS)-stimulator of interferon genes (STING) signalling axis, triggering an innate immune reaction[105]. Nuclear DNA (including extranuclear chromatin) could be released to the cytoplasm due to impaired nuclear envelope integrity, nuclear envelope blebbing, or nuclear export processes[105]; mitochondrial DNA could be leaked to the cytoplasm due to mitochondrial damage and inefficient elimination of damaged mitochondria via mitophagy[101,102]. The cGAS-STING signalling axis detects these DNA fragments to initiate an innate immune reaction, linking it to autoimmunity, inflammation, senescence, and autophagy[105]. Collectively, genomic instability, accumulation of mitochondrial DNA leaked into the cytoplasm andincreased levels of cellular stress granules, are linked to inflammation, accelerated ageing and a broad range of neurodegenerative diseases[95,96,101]. Although maintenance of DNA/RNA homeostasis is critical for healthy ageing, the contribution of RNAphagy and DNAphagy to long-term tissue health requires further exploration.

3.3. Autophagy of sub-cellular organelles: mitophagy, ER-phagy, nucleophagy, lysophagy

Ageing is associated with an accumulation of damage to cellular organelles. The timely and efficient disposal and recycling of dysfunctional organelles is necessary to maintain cellular function and viability. Selective autophagy is the common mechanism underlying the clearance of sub-cellular dysfunctional and/or superfluous organelles, such as, mitochondria (mitophagy), endoplasmic reticulum (reticulophagy or ER-phagy), nucleus (nucleophagy) and lysosomes (lysophagy)[17]. Both membrane-bound and soluble selective autophagy receptors are involved in the selective degradation of damaged or surplus organelles[18,106].

Among the different types of autophagy targeting sub-cellular organelles, the best investigated is mitophagy. Mitophagy is the selective autophagic degradation of damaged or surplus mitochondria. The PINK1-PARKIN mediated pathway for degradation of heavily depolarised mitochondria is best understood and involves Ser-65 phosphorylated ubiquitin that attracts soluble selective autophagy receptors NDP52, optineurin and p62, which then recruit the core autophagy machinery for autophagosome formation on the damaged mitochondria. In addition, other basal-, developmental or stress-induced mitophagy pathways involve binding to a series of LIR-containing mitochondrial outer membrane proteins, such as NIX (BNIP3L), BNIP3, FKBP8, FUNDC1, BCL2L13, PHB2, AMBRA1 and also LC3-binding mitochondrial lipids like cardiolipin[29] (**Fig. 1d**, 1st pathway). While whole mitochondria can be degraded via standard mitophagy, it appears that organelles with minor damage can be 'repaired' by other quality

control mechanisms such as the piecemeal mitophagy pathway, which is a basal housekeeping mitophagy pathway that involves degradation of mitochondrial proteins in an LC3C- and p62-dependent manner[107] (**Fig. 1d**, 2nd pathway). In addition, other mitochondrial degradation pathways include the mitochondria-derived vesicle (MDV) pathway, where damaged cargo (e.g., impaired mitochondrial proteins) are delivered to the lysosome for degradation in a process dependent on Syntaxin-17, PINK1 and Parkin[108] (**Fig. 1d**, 2nd pathway). A recent study in *C. elegans* shows that damaged subcellular components, including mitochondria among others, can be budded off from certain neurons via membrane-bound vesicles (termed 'exophers')[109]. Once in the extracellular space, these damaged organelles can be engulfed and digested by surrounding cells[109]. This cellular release of 'exophers' is also conserved in mammals, as cardiomyocytes release exophers (containing mitochondria) to be received and eliminated by adjacent macrophages[110].

Accumulating evidence highlights that mitophagy is a critical contributor for cellular physiology and organ homeostasis. First, there is an increase of mitophagy from juvenile to adulthood, followed by a dramatic reduction in old animals. For example, there was an increase of basal mitophagy in fly flight muscle from the ages of 1 week to 4 weeks)[111]; in mice, mitophagy in the dentate gyrus (DG), a region that is essential for memory, was reduced by approximately 70% between 3 and 21-months of age[112]. Mitophagy is also impaired in high-fat feeding conditions[112] and in neurodegenerative diseases (reviewed in[29]). Indeed, mitophagy is reduced in mice with AD (by approximately 50% in the hippocampus vs. healthy controls)[113], PD (reviewed in[114]) and HD (by over 70% in the DG region of HTT expressing mice vs WT controls)[112]. Second, intact mitophagic machinery is required for longevity. Since there are several redundant mitophagy pathways, dysfunction of isolated individual mitophagy pathways may not affect lifespan [115,116]. However, mitophagy is essential for longevity under conditions of low insulin/IGF-1 signalling (*daf-2* mutant worms), caloric restriction (*eat-2* mutants) or mitohormesis[115,117], as well as for the maintenance of neuronal functions in response to stressful conditions[101]. Third, mitophagy induction is sufficient to improve healthspan and extends lifespan in several model organisms and rescues age-associated neurodegenerative phenotypes in AD[113,118] and prolongs lifespan in nematode and fly models of accelerated ageing[93,119,120]. Moreover, functional mitophagy is essential for restraining innate immunity as mitochondrial stress can lead to the release of damage-associated molecular patterns (DAMPs) that activate innate immunity. Inflammation resulting from excessive exercise in *Pink1* and *Parkin* knockout mice was shown to be suppressed by loss of STING, a central regulator of the type I Interferon response to cytosolic DNA[101].

Other autophagic pathways that target subcellular organelles include ER-phagy, nucleophagy, and lysophagy. In yeast, Atg39 regulates perinuclear ER-phagy and nucleophagy, while Atg40 is necessary for cortical/cytoplasmic ER-phagy[121] (**Fig. 1e**). Nucleophagy is conserved in mammalian cells[122] with a nuclear LC3-lamin B1 interaction-based nuclear-to-cytoplasmic degradation, which may be a guarding mechanism protecting cells from tumorigenesis[123] (**Fig. 1f**). Lysophagy is regulated by both ubiquitin-dependent (Galectin-3-TRIM16-ULK1/ATG16L-autophagy receptor-LC3, the F-box protein FBXO27 and UBE2QL1) as well as -independent (Galectin-8-autophagy receptor-LC3) pathways (reviewed in[124]) (**Fig. 1g**). Maintenance of functional and effective lysosomes, via timely and efficient lysophagy, is essential for cell survival. In particular, dysfunction in lysosome membrane proteins such as SCAV-3, the *C. elegans* homolog of human LIMP-2, has been linked to reduced lifespan, implicating lysosome integrity as a defining factor of longevity[125,126 20]. Moreover, dysfunctional lysosomal membrane proteins coupled to leakage of proteolytic enzymes (i.e., cathepsin D) into the cytosol has been associated with ageing and pathological ageing in a broad range of neurodegenerative diseases[127]. Thus, maintaining physiological lysophagy is critical for many cellular processes and is presumably important for health and longevity, as lysosomal rupture triggers endo-lysosomal damage responses, and even lysosomal cell death, which links to ageing and diseases[127,128].

Collectively, imbalanced quality surveillance system of sub-cellular organelles, such as mitochondria, ER, small nuclear fractions, and lysosomes, might be a causative factor for age-related pathologies as well as premature ageing. Further studies on mitophagy, ER-phagy, nucleophagy, and lysophagy deciphering their multi-layer regulatory network and their association with ageing and health are necessary. In particular, studies to address how these processes change with age, and how they influence age-related tissue function will lead to provide critical insights with broad relevance to human health and quality of life.

3.4 Xenophagy

Xenophagy (from the Greek meaning "to eat foreign matter") is the process by which autophagy targets pathogens[129]. Many pathogens are known to be degraded by autophagy, while others take over core autophagy components for their own benefit[130] (**Fig. 1h**). Indeed, several studies have revealed that autophagy can target bacteria like *Rickettsia conorii*[131], *Listeria monocytogenes*[132] *Streptococcus pyogenes*[133], and *Mycobacterium tuberculosis*[134,135]. Xenophagy may also protect the body against invasion by viruses and parasites.

Upon intake by inhalation, alveolar macrophages capture *Mycobacterium tuberculosis*. However, this bacterium has evolved to be able to impair phagosome maturation (which in normal conditions would lead to phagocytosis), and ends up hijacking the macrophage[136]. Later on, using ESX-1 (6 kDa early secretory antigenic target/ESAT-6 secretion system 1) secretions, the bacterium is able to break free from the phagosome and enters the cytosol. Here, xenophagy comes into action. cGAS detects the bacterial DNA[137], which results in ubiquitination of the invading bacteria by Smurf1 (or Parkin)[138]. NBR1 (or p62) attaches to these ubiquitin chains, resulting in the recruitment of LC3B, and finally autophagic degradation occurs[139]. Indeed, the absence of autophagic machinery components namely, ULK1[140], BECN1[141], p62[142], ATG7[143] and TBK1[142] may promote the proliferation of the bacterium. The mechanism is similar for specific viruses. Beclin 1, p62, and selective autophagy of viral capsids can be protective against Sindbis virus[144,145]. However, other viruses, such as herpes simplex virus type 1, have evolved to inhibit autophagy by targeting BECN1[146]. In addition, several studies have highlighted the importance, and possible therapeutic relevance, of autophagy for controlling SARS-CoV-2, the coronavirus that causes COVID-19 [147-149]. Regarding parasites, autophagy can control *Toxoplasma gondii*. Knockout of ATG5, ATG7 or ATG16L1 renders mice more susceptible to succumb to parasites[150]. A detailed review between parasites and autophagy is available[151].

Although there is not much data available on a direct link between xenophagy and ageing or lifespan, it is conceivable that blocking the infection of exogenous intruders is required for maintenance of a healthy state and reduced inflammation[126,152]. Further work to investigate the molecular mechanisms of xenophagy and their association with ageing and longevity is required.

4. Defective autophagy in diseases associated with accelerated ageing, neurodegenerative diseases and inflammaging

Accumulating evidence from studies using laboratory animals and human samples supports an essential role for autophagy in embryonic development, tissue health and lifespan through the suppression of age-associated inflammation (inflammaging), maintenance of genomic integrity, preservation of cellular/tissue homeostasis and rejuvenation of stem cells(**Fig. 2a**, references[13,14,103,153]). While autophagy is tightly regulated by multiple molecular pathways involving central modulators of energy metabolism, such as AMPK, mTORC1, sirtuins, and calcineurin (**Fig. 2b**), several interventions such as dietary restriction, exercise, and supplementation of small chemical compounds (detailed below) stimulate autophagy[153] . Recent preclinical studies link impaired general autophagy or subtypes of autophagy (in some cases with no change of or even increased macroautophagy) to pathological states, such as

progeria[103] (**Fig. 2c**), neurodegenerative diseases[29,30] (**Fig. 2d**), and other disorders[13,14,103,153] (**Fig. 2e**).

Understanding the relationship between compromised autophagy and other hallmarks of ageing will provide a better understanding of the molecular events that promote ageing and disease[14,103,153]. Among the many age-related changes previously described, inflammation is linked to autophagy and has emerged as a major driver of age-related tissue damage. [53,153-155]. Inflammation is an evolutionary conserved protective mechanism designed to maintain organismal homoeostasis against acute and local perturbations, and serves as an adaptive response to infection or injury [156]. Chronic, systemic inflammation develops progressively with age and contributes to organismal deterioration through a process termed "inflammaging"[155].

Autophagy has been identified to be amongst the pivotal pillars that orchestrates the differentiation and metabolic state of innate immune cells. In particular, the balance between mTOR and AMPK activation plays a central role in immune cell maintenance and function. Upon mTOR activation, autophagic flux is reduced, accompanied by increased cellular glycolytic activity, giving rise to proliferative and pro-inflammatory phenotype of macrophages. In contrast, AMPK activation drives autophagy and promotes the OXPHOS-dependent function of non- or anti-inflammatory macrophages[157].

Autophagy also regulates the NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome, which is an intracellular protein complex that activates caspase-1, which in turn catalyses the cleavage, activation and subsequent release of pro-inflammatory cytokines (i.e., IL-1β) that can induce neurodegeneration[155,158]. The NLRP3 inflammasome has been identified as a critical component of the innate immune response (i.e., microbial motifs, endogenous danger signals and environmental irritants) and orchestrates host immune homeostasis[158]. Defective autophagy, for example in models of selective knockout or knockdown of genes/proteins involved in the autophagic core machinery (i.e., ATG5, ATG7, BECN1 and MAP1LC3B), results in unrestricted inflammasome activation and consequent inflammation. Likewise, promotion of autophagy through starvation or pharmaceuticals (i.e., rapamycin) inhibits the inflammasome[159]. In addition, evidence stemming from an APP/PS1 mouse model of AD demonstrated mitophagy-induced inhibition of the NLRP3 inflammasome, thus reducing neuroinflammation[113]. These findings imply an important role for autophagy in the regulation of inflammation, and in turn, ageing and neurodegenerative diseases.

5. Anti-ageing effects of autophagy modulators

The mounting evidence that an imbalance of autophagy is an important age-associated characteristic has driven extensive research into the development of compounds that can promote autophagy[1]. Pharmacological agents promoting autophagy could be classified based on their effect on the mTOR pathway[160]. mTOR inhibition by rapamycin has been shown to reduce protein synthesis and promote autophagy, both of which contribute to extended lifespan in yeast, nematodes, flies and mice (**Table 3**). In addition, rapamycin has been demonstrated to protect against neurodegenerative diseases, including AD, via promotion of autophagy; however, rapamycin treatment was observed to be detrimental in the case of models of amyotrophic lateral sclerosis (ALS), possibly due to non-autophagy-related side effects[160]. Other pharmacological agents reported to promote autophagy via direct interaction with mTOR include Torin-1 and PP242[161]. The mTOR-independent promoters of autophagy mainly act via the AMPK pathway. Examples include metformin and trehalose, which have been demonstrated to be effective in enhancing autophagy, extending lifespan and protecting against neurodegeneration in experimental models[160].

Compounds such as resveratrol and spermidine modulate the acetylation state of proteins to regulate autophagy and promote longevity. Resveratrol is a natural polyphenol that reportedly promotes lifespan in *C. elegans* and healthspan in mice via activation of NAD^+-dependent

deacetylase, sirtuin-1 (SIRT1)[94,162,163]. Spermidine is a polyamine that extends the lifespan of yeast, worms, flies and mice via enhancing autophagy through inhibition of EP300 (E1A-binding protein p300) acetyltransferase[164], among other mechanisms[47,165-167]. The longevity-extending effects of spermidine are abolished upon depletion or deletion of essential autophagy genes such as *bec-1*/*BECN1* in *C. elegans* and *Atg7* in yeast and flies[166,168].

Other small molecules that induce subtypes of autophagy, especially mitophagy, also enhance longevity and suppress age-associated diseases. These include NAD^+, a fundamental metabolite in energy metabolism, redox homeostasis, mitochondrial function and the arbitration of cell survival and death[154]. NAD^+-activated SIRTs stimulate autophagy via mTOR inhibition and deacetylation of several key autophagy proteins (ATG5, ATG7 and MAP1LC3B) [169,170]. In addition, the NAD^+-SIRT axis activates mitophagy by increasing the activity of a series of mitophagy-related proteins, such as PINK-1, Parkin, NIX (in *C. elegans* DCT-1) and BNIP3[119,171]. Supplementation of WT worms, flies and mice with NAD^+ precursors, such as nicotinamide (NAM), nicotinamide riboside (NR), or nicotinamide mononucleotide (NMN), can increase lifespan and/or improve healthspan[93,172-174]. NAD^+ augmentation also prevents memory loss in both Aβ and Tau animal models of AD in a mitophagy-dependent manner (requiring *pink-1*, *pdr-1*, or *dct-1*)[113]. Over seven clinical trials have shown the safety and bioavailability of NR (1 -2 g/day for up to 3 months), and more than 30 clinical trials on the use of NR to treat premature ageing and other age-related diseases (see review in[154]). Another clinically promising mitophagy inducer is Urolithin A (UA), a metabolite of ellagitannins from the gut microflora. UA extends healthspan and lifespan in *C. elegans*, with lifespan extension depending on genes involved in autophagy (*bec-1*, *sqst-1*, *vps-34*) and mitophagy (*pink-1*, *dct-1*, and *skn-1*)[175]. Intriguingly, UA was able to inhibit memory loss in both Aβ and Tau animal models of AD in a mitophagy-dependent manner (*pink-1*, *pdr-1*, or *dct-1*)[113]. UA (500 mg and 1,000 mg /day for 4 weeks) was also shown to be safe in a stage I clinical trial[176]. A summary of different lifespan/health-benefit mitophagy inducers can be found in **Table 3**. Encouraged by the clinical safety of NR and UA, their effects on healthspan and lifespan in the elderly deserve further investigation. Despite recent progress in the identification of novel as well as well-known autophagy-inducing compounds, it is also of great importance to highlight the pleiotropic effects of these pharmacological interventions and to completely understand the full complement of targets that they interact with in order to use them safely for therapeutic intervention.

While experimental/empirical evidence indicates that autophagy is defective in the elderly, it is conceivable that exposing individuals to autophagy inducers, dietary restriction, and exercise late in life could boost autophagy and result in benefits to tissue function [177,178] (**Fig. 3a**). Based on preclinical data, it is presumed that autophagy stimulation (ideally to increase autophagy to the levels observed early in adulthood) may be sufficient to provide benefits (**Fig. 3b**).

6. Conclusions and future perspectives

Mounting evidence from studies using laboratory animals, human tissues, and related clinical trials support that: a) there is an age-dependent decline of autophagy, b) autophagy is a crucial determinant of cellular health and organismal longevity and c) impairment/imbalance in autophagy promotes pathological ageing and disease. Given the broad spectrum of unique properties associated with autophagy, we propose ‘compromised autophagy’ is a central feature of normal ageing, and that the term ‘autophageing’ could be used to describe the diverse ways in which autophagy regulates long-term cellular health. Although the relationship between autophagy and ageing is often described as “decreased autophagy is detrimental” and “increased autophagy is beneficial, this may be too simplistic a picture. Instead, long-term health benefits will likely arise from achieving the right balance of autophagy, which itself will depend upon the tissue and organismal age. For example, in *C. elegans*, impairing autophagy early in life has a negative effect on longevity, whereas impairing autophagy in adulthood can have beneficial effects on lifespan[51]. Similarly, increased autophagy through a hypermorphic

allele of *atg-5,* has differential effects on polyglutamine aggregation in muscles and neurons of *C. elegans*[179]. In flies, a mild increase in autophagy extends lifespan, while strongly increasing autophagy shortens lifespan[180]. It should also be noted that autophagy induction may also result in unwanted effects, such as multiple senescent pathologies[51] and resistance to cancer therapy (reviewed in [181]). Collectively, these observations suggest that the level and balance among the different forms of autophagy in each tissue is highly specified for each stage of life and an understanding of this will be crucial for healthy ageing. Thus, while different types of autophagy may influence ageing to different extents, a goal will be to find approaches to promote the right levels of autophagy, at the right time, in the right tissues to enhance health (**Fig. 3**).

To this end, there remain many outstanding questions related to 'autophageing' that need to be addressed. What are the intricate mechanisms that orchestrate distinct autophagy pathways? How is autophagy spatially- and temporally-regulated and how does the disruption of this regulation suppress or promote disease? Are some aspects of autophagy more important in an age- and/or tissue-dependent manner? What are the determining factors that dictate the route of degradation via the UPS or autophagy? How are clearance mechanisms balanced with synthesis and folding through the proteostasis network? What are the thresholds of life-benefit and life-detrimental autophagy? In line with the traditional Chinese 'Yin-Yang' philosophy, autophagy must be balanced, as diminished autophagy results in the accumulation of toxic subcellular components while excessive autophagy can lead to organ atrophy[14,29,51,153]. Finally, are there any conditions or diseases where we should be cautious about inducing autophagy in that protection against one form of pathology increases the risk for another? E.g., pancreatic cancer cells may hijack autophagy processes to obtain nutrients for growth; hence, in this condition autophagy inhibition in combination with cancer chemotherapies may inhibit pancreatic cancer growth[182,183]. Addressing these questions will facilitate our understanding of the ageing process and, more importantly, enable us to identify novel targets that may be manipulated for therapeutic intervention in age-associated diseases.

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Declaration of Interests
E.F.F. has CRADA arrangement with ChromaDex, and is consultant to Aladdin Healthcare Technologies, Vancouver Dementia Prevention Centre, and Intellectual Labs. AKS is a consultant to Oxford Healthspan. D.C.R. is a consultant for Aladdin Healthcare Technologies Ltd, Drishti Discoveries and Nido Biosciences.

Figures & Legends

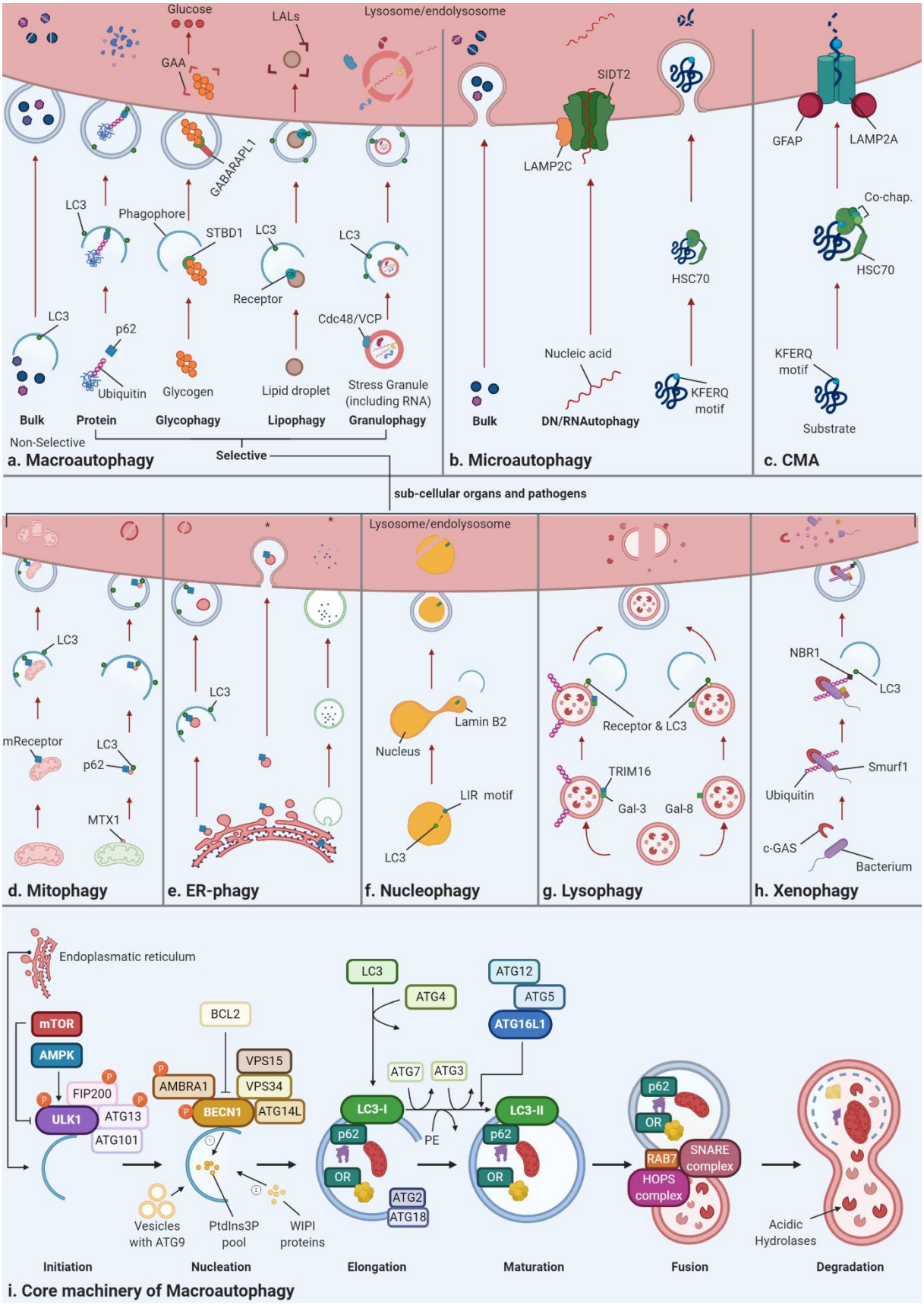

Figure 1. Different mechanisms of autophagy. (a) Macroautophagy: The non-selective process of macroautophagy will target macromolecules or sub-cellular organelles in bulk. An autophagosome engulfs bulk material and delivers it to the lysosome (or endo-lysosome) for degradation. Targeting on proteins: Here we present an example of aggrephagy. Aggregates of proteins are ubiquitinated. These chains of ubiquitin are targeted by p62 and NBR1 (green), which bind to LC3 for the formation of an autophagosome. This is delivered to a lysosome (or endo-lysosome) for degradation. Glycophagy: It begins by STBD1 (Genethonin-1) binding to glycogen, then, the glycogen attaches to GABARAPL1 (orange) and is delivered to the phagosome. The phagosome fuses with the lysosome, where glycogen breaks down into non-phosphorylated glucose by enzymes like glycogen-hydrolyzing acid α-glucosidase (GAA). Lipophagy: An engulfment of the lipid droplet occurs with the support of a specific receptors in blue (to be determined). These autophagosome transport the lipids to the lysosome where they are degraded into free fatty acids, which are then converted into ATP. Degradation of RNA via granulophagy: The degradation of stress granules (RNA + proteins) is mediated by Cdc48/VCP, which assists in the engulfment of the granule. This allows the stress granule to be delivered to the lysosome for degradation. **(b)** Microautophagy: The process of direct invagination by the lysosome can be non-specific (bulk), which occurs by direct intake of lysosomes. For specific microautophagy, nucleic acids (DNA/RNA) bind to the receptor LAMP2C (orange) which also binds to lysosomes. This process allows nucleic acids to be taken by the lysosomes. It has been proposed that a transporter called SIDT2 (green) might play a role in direct uptake of nucleic acids by the lysosomes as well. It can also occur that the chaperone HSC70 targets the pentapeptide motif KFERQ of proteins, then, with the company of unknown cochaperones, it brings it to the lysosome by binding it. In mammals, the degradation process of microautophagy has been observed in late endosomes rather than in lysosomes (endosomal microautophagy). **(c)** Chaperone-mediated autophagy (CMA): The mechanism also involves the targeting of the KFERQ pentapeptide motif by HSC70, in addition, other cochaperones such as HSP40 may support the targeting. Then, through the receptor LAMP2A, the substrate and the chaperone/cochaperones are taken into the lysosome for further degradation. This receptor is modulated by the glial fibrillary acidic protein (GFAP). **(d)** Mitophagy: Damaged mitochondria can be fully engulfed by mitophagy (left). Different specific-mitophagy receptors can bind to the mitochondrion, which bind to LC3. Later, engulfment occurs, the mitochondrion is delivered by the autophagosome to a lysosome for degradation. Piecemeal mitophagy (right) allows protein components of the mitochondrion to be degraded. As an example, MTX1 is targeted by LC3, this binding results in the recruitment of p62. An autophagosome is formed. This is then delivered to a lysosome for fusion and further degradation of MTX1. **(e)** ER-phagy (e1.): Macro-ER-phagy in mammals uses the specific receptors FAM134B, RTN3L, ATL3, SEC62, CCPG1 & TEX264, which are located in different places in the ER. These receptors bind to LC3, forming an autophagosome which binds to a lysosome to degrade the ER. (e2.): Micro-ER-phagy in mammals uses the receptors SEC62 and is taken by invagination by the lysosome/endolysosome. (e3.): Vesicular-delivery uses the receptor FAM134B. Here, the receptor is detected by a vesicle, which brings misfolded proteins from the ER to the lysosome for degradation. *These two processes do not constitute part of macroautophagy due to their lack of phagophore. **(f)** Nucleophagy: In mammals, when nucleophagy is triggered, nuclear LC3 binds to the LIR motif of lamin B1. These form a bulge which is pinched off to the cytoplasm where degradation by autophagy will occur. **(g)** Lysophagy: Two ways this can be achieved is with or without ubiquitination. Galectin-3 (Gal-3) can bind to target lysosomes. It recruits the protein TRIM16 to form the TRIM16-Gal3 complex. These two will recruit autophagic proteins like ULK1 and ATG16L1. Furthermore, ubiquitination occurs on the lysosome, resulting in the recruitment of p62. This protein binds to LC3 to further continue with the autophagic process. Meanwhile, an ubiquitin-independent process can occur with a different sensing protein, Galectin-8 (Gal-8), which is capable of directly binding to the receptor NDP52, which binds to LC3 and continues the autophagic process. (h) Xenophagy: A bacterium's DNA is detected by c-GAS, sensors which trigger a process of ubiquitination via Smurf1. This is followed by the attachment of NBR1 to the ubiquitin chains. The receptor NBR1 binds to LC3 in order to continue with the autophagy

process for the degradation of the bacterium. **(i)** Core machinery of Autophagy: The initiation of autophagy initiate with ULK1, mainly mediated by AMPK and mTOR. The ULK1 stimulates the Beclin 1 complex (PI3KC3 complex), producing a pool of PtdIns3P, which recruits WIPI proteins. From elongation to maturation, LCR goes through two main conversions. Meanwhile, p62 or OR (other receptors) identify cargos for degradation. Finally, fusion occurs mainly by the assistance of Rab proteins, SNARE proteins and a HOPS complex. After fusion, the degradation process occurs and the cargo is degraded.

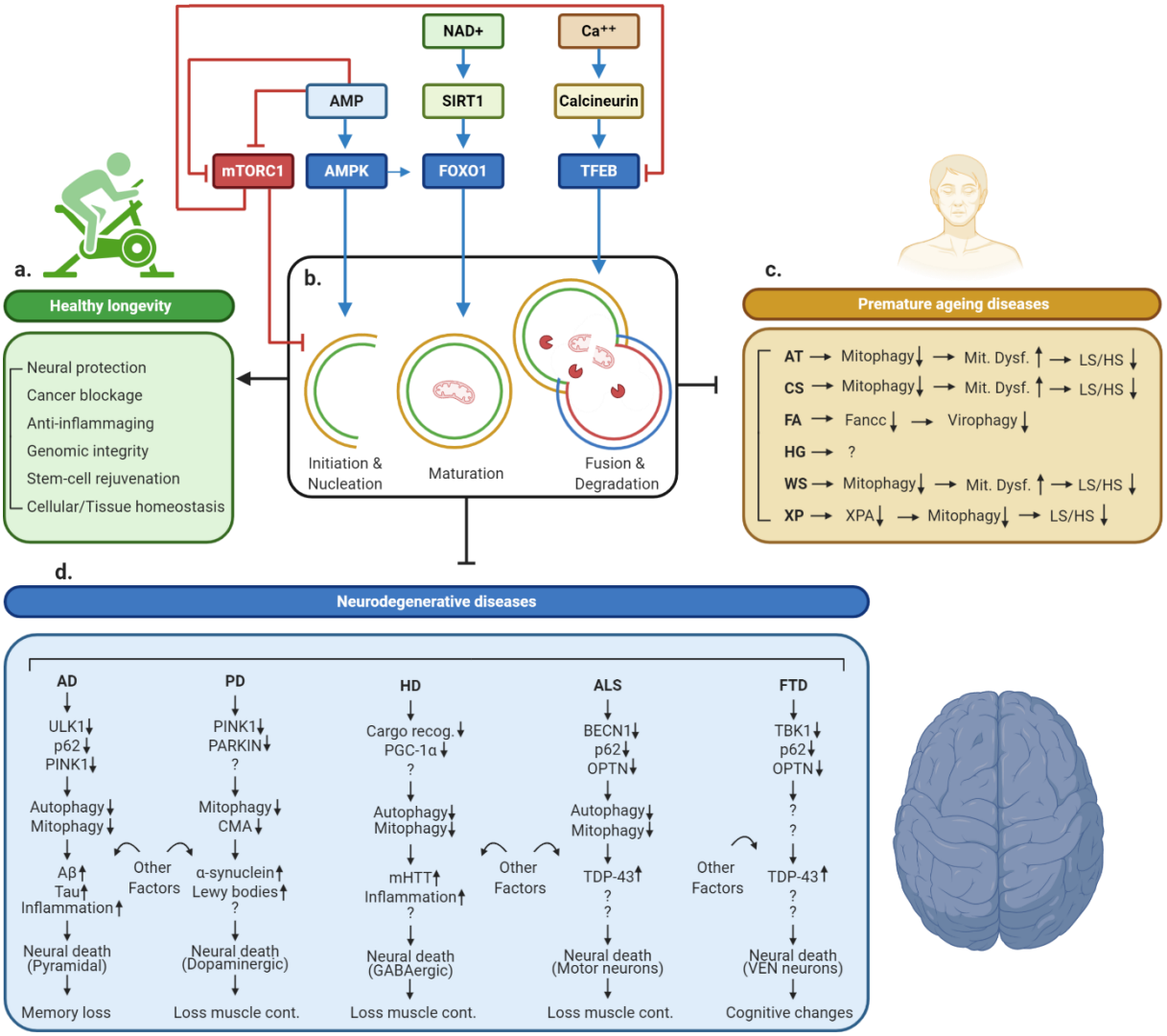

Figure 2. Autophagy in health and disease. (a) Autophagy participates in multiple processes that are essential for a healthy longevity. **(b)** A brief map of some of the major known mechanisms that regulate autophagy and their influences in the process. **(c)** A list summarizing premature ageing diseases with impaired mitophagy as a cause of mitochondrial dysfunction, which contributes to short lifespan (LS) and healthspan (HS). These premature ageing diseases are Ataxia Telangiectasia (AT), Cockayne Syndrome (CS), Fanconi Anemia (FA), Hutchinson-Gilford Syndrome (HG), Werner Syndrome (WS), Xeroderma Pigmentosum (XP, especially group A). Changes of autophagy and mitophagy in HG are elusive. **(d)** Autophagy (including sub-types of selective autophagy, like mitophagy) are impaired in broad neurodegenerative diseases, contributing to disease progression. Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD), Amyotrophic Lateral Sclerosis (ALS), and Frontotemporal Dementia (FTD). We emphasize that these are not the only drivers of the disease and other processes may play roles leading the pathology and symptomatology. '?', information unknown.

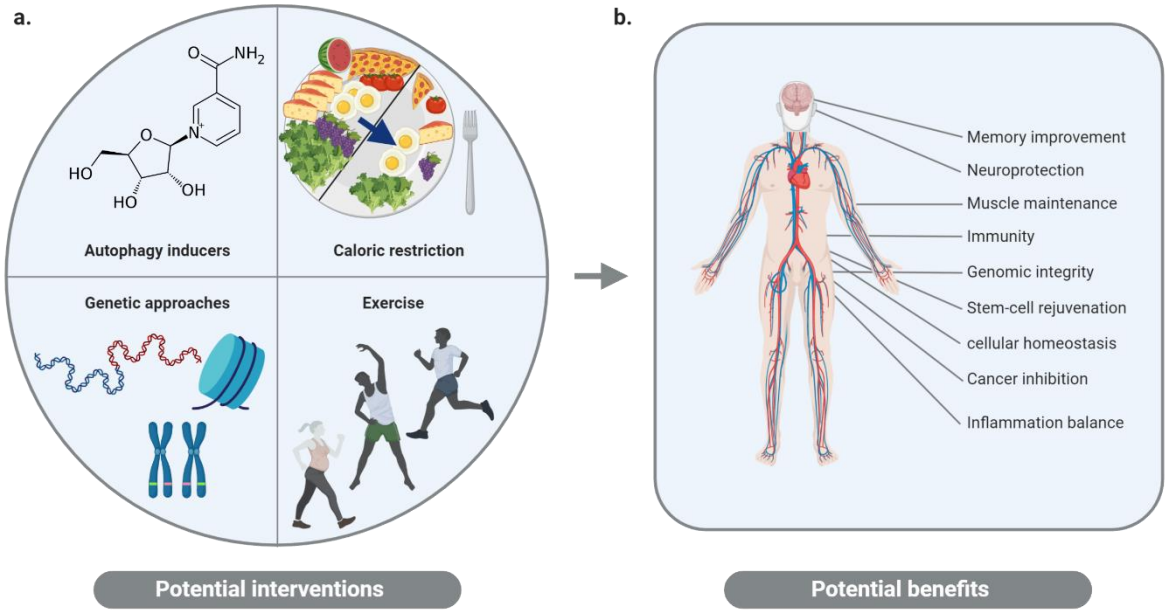

Figure 3. Autophagy maintenance during ageing via approachable procedures to achieve healthy longevity. (a) Potential interventions to stimulate autophagy: autophagy inducers, dietary restriction, exercise, and genetic approaches. **(b)** Autophagy induction could positively impact human health.

Table 1. A summary of autophagy genes/proteins involved in anti-ageing and healthy longevity.

Protein (names in different species)	**Functions**	**Modification effect in longevity**
ATG1 (Y & F), UNC51 (W), ULK1 (M), ULK1 and ULK2 (H)	Kinase required for the formation of the autophagosome[184].	(W) Mutants age faster[32]. (F, Y, W) Essential for longevity when using approaches such as TOR suppression, ↑AMPK, dietary restriction, rapamycin and others[32,37,185,186].
ATG2 (Y, W & F), ATG2A/B (M & H)	Lipid transport protein crucial for the formation of the autophagosome[187].	↓ reduces lifespan[188]. (F) Significantly decreases with age[25].
ATG4 (Y), ATG4.1 (W), ATG4A (F), ATG4B (M & H)	Protease required for conjugation/deconjugation of Atg8 proteins to phosphatidylethanolamine[189].	(W) essential for longevity when using approaches such as mir-34 loss-of-function[190].
ATG5 (Y, W, F, M & H)	Part of the E3 complex required for Atg8 lipidation[191].	(M) ↑ ubiquitously increases lifespan[36]. (F, Y) Essential for longevity when using approaches such as methionine restriction and rapamycin[192,193].
Y: Vps30/Atg6 W: BEC-1 F: Atg6 M: BECN1/Beclin 1 H: BECN1/Beclin 1	Subunit of the class III PI3K complex required for autophagosome formation[191].	(F, W, Y) certain mutants live shorter lifespan and age faster. Essential for longevity when using approaches such as TOR suppression, *mir-34* loss-of-function, treatment by spermidine and urolithin A, and dietary restriction[32,168,175,190,194,195].
ATG7 (Y, W, F, M & H)	E1 enzyme required for Atg8 lipidation[191].	(M, W) ↓ or absence of it reduces lifespan, increases atrophy and inflammation[196,197]. (F, W, Y) important for longevity when using approaches, such as spermidine, dietary restriction and methionine restriction[168,192,194]. (H) significantly reduced in the muscle of sarcopenic adults[196]. (M) significantly reduced in the muscles of older mice[196].
ATG8F (Y), LGG1 (W), ATG8A (F), LC3 (M & H)	Small ubiquitin protein conjugated to PE in autophagic membranes. Interacts with protein containing AIM/LIR motifs.	(F) ↑ in neurons increases lifespan[25]. (F) ↑ in muscles increases lifespan[198]. (F) ↓ produces neurodegeneration and reduces lifespan[25]. (Y) essential for longevity when using approaches such as methionine restrict (F) Crucial for the formation of the autophagosome. At week 4, it is downregulated up to 60%[25].ion[192].
ATG9 (Y, W & F), ATG9A (M & H)	Transmembrane protein required for autophagosome formation[199].	(W) essential for longevity when using approaches such as mir-34 loss-of-function[190].
ATG12 (Y, W, F, M & H)	Forms a complex with ATG5 and ATG16L1[200].	(W) ↓ reduces lifespan[197].
ATG15 (Y & H)	Required for the lysis of subvacuolar vesicles[201].	(Y) essential for longevity when using approaches such as caloric restriction[202].
ATG18 (Y & W), ATG18a (F), WWIPI1-4 (M & H)	PtdIns3P binding proteins essential for autophagy[203].	(W) mutants age faster and loss of function reduces lifespan[23,32]. Its expression in the neurons and intestine is essential for maintaining wild type lifespan[204]. Inhibiting S6K increases its levels[205]. Essential for longevity when using approaches such as TOR suppression and dietary restriction[32,204]. (F) Significantly decreases with age[25].
Parkin (W, F, M & H)	Ubiquitin E3 ligase that ubiquitinates outer mitochondrial membrane (OMM) proteins, promoting mitophagy[206].	(F) ↑ ubiquitously and in neurons increases lifespan.
PINK1 (W, F, M & H)	Mitochondrial kinase that phosphorylates ubiquitin and recruits Parkin upon mitochondria depolarization[207].	(W) essential for longevity when using multiple approaches, such as NR and UA treatment among others[115,175,208].

SQST1 (W), REF2P (F), P62 and NBR1 (M), P62/SQSTM1 and NBR1 (H).	Ubiquitin-binding autophagy receptor involved in selective autophagy[209].	(W) ↑ increases lifespan[62,63]. Essential for longevity and for mitophagy inducers (e.g., UA)-induced lifespan/healthspan improvement[62,175,208].
HLH-30 (W), MITF (F), TFEB (M & H)	Transcription factor for genes involved in autophagy, lysosome, and phagocytosis[39].	(W) ↑ increases lifespan[39]. Inhibiting S6K increases its levels[205].
VPS34 (Y, W, M), Pi3K59F (F), PIK3C3 (H)	PI3K required for autophagosome formation[210].	(W) essential for longevity when using multiple approaches, such as UA treatment and dietary restrictions[175,195].

Abbreviations: Yeast (Y), Worms (W), Flies (F), Mice (M), Humans (H), ↑ (Overexpression), ↓ (Reduced expression), urolithin A (UA), nicotinamide riboside (NR).

Table 2. Lists of accelerated ageing and neurodegenerative diseases with aetiologies/disease initiation or progressions liking to impaired autophagy

Categories	Disease	Relations to autophagy
Accelerated ageing	Ataxia Telangiectasia	ATM participates in both autophagy and mitophagy; *Atm* mutation leads to defective mitophagy[119,120].
	Cockayne syndrome	CSB participates in autophagy/mitophagy[119,211].
	Fanconi Anemia	Dysfunction of different Fanconi Anemia protein members lead to dysfunctional xenophagy and mitophagy[212].
	Hutchinson-Gilford syndrome	p62 interacts with progerin, and boosting autophagy with rapamycin improves clearance of progerin[213].
	Werner syndrome	Werner participates in mitophagy[93].
	Xeroderma Pigmentosum	XPA participates in both autophagy and mitophagy; *XPA* mutation leads to defective mitophagy[119].
Neurodegeneration	Alzheimer's Disease (AD)	The familial AD gene *PS1* mutant disrupts autophagy and lysosomal proteolysis[214]. Impaired mitophagy in postmortem brain tissues from AD patients and in both Aβ and Tau animal models of AD[113]. Proteins involved in autophagy or mitophagy, including PINK1, TBK1 (p-), ULK1 (p-), OPTN, BECN1/Beclin 1, AMBRA1, Bcl2L13, FUNDC1, MUL1, BNIP3L/NIX, were reduced in some AD patient samples (postmortem brain tissue or IPSC-derived cortical neurons)[113]. Reduced BAG-3 in the entorhinal cortex of AD[68].
	Parkinson's Disease (PD)	(M) α-Synuclein, from Lewy bodies, affect the localization of ATG9[215]; α-Synuclein is also capable of impairing the chaperone-mediated autophagy pathway[216]; Overexpression of ATG6 ameliorates the aggregation of α-syn[217]; *PINK1* and *PARK2* mutations lead to familial PD (reviewed in[29]) .
	Amyotrophic Lateral Sclerosis (ALS)	(H) Mutations of many autophagy genes, such as *TBK1* and *SQSTM1*, link to ALS (reviewed in[29])
	Frontotemporal Dementia (FTD)	(H) Mutations of many autophagy genes, such *TBK1* and *SQSTM1*, link to ALS (reviewed in[29])
	Huntington's Disease (HD)	*ATG7* variants may pay a role in the age of onset[218,219]. Cargo recognition is disrupted[220]. Huntingtin mutant may be sequestering ATG6[221]. Absence of Alfy disrupts autophagy and accelerates aggregation[222].
	Autosomal Recessive Juvenile Parkinsonism (ARJP)	(H) *PARK2* mutation plays a causative role[223].
	Ataxia	(H) ATG5 mutation leads to ataxia[224].
	Hereditary Spastic Paraparesis (HSP)	(H) TECPR2 mutation causes some types of HSP[225]. SPG15 mutation may cause some types of HSP[226].
	Lafora Disease	There are correlations between lack of laforin and an increase in TOR activity, inhibiting autophagy[227].
	SENDA & BPAN	(H) WDR45 mutations have been suggested to cause it[228,229].

Abbreviations: Ataxia Telangiectasia mutated (ATM), Mice (M), Cockayne Syndrome group B (CSB), Xeroderma Pigmentosum group A (XPA), Humans (H), ↑ (Overexpression), ↓ (Reduced expression), Static encephalopathy of childhood with neurodegeneration in adulthood (SENDA), Beta-propeller protein-associated neurodegeneration (BPAN).

Table 3. Summary of a list of autophagy inducers which extend healthspan and increase lifespan in laboratory animals

Pharmacological Agents	Health benefits	Modes of action
Metformin	↑lifespan (W, M), healthspan	activates AMPK and other mechanisms[230] (also reviewed in [231])
Rapamycin	↑lifespan (W,F,M), different healthspan parameters (F,M)	Directly autophagy induction via mTOR inhibition (reviewed in [231])
Resveratrol	↑lifespan (Y,W,F,M[1]), different healthspan parameters (M)	SIRT1-dependent induction of autophagy, and non-autophagy pathways[94] (also reviewed in[103])
Spermidine	↑Median lifespan (W,F,M), different healthspan parameters (M, R)	Autophagy, anti-inflammation, arginine and NO metabolism[165,168]
NR/NMN	↑lifespan (W, F, M), healthspan (W, F, M), memory (M)	Autophagy/mitophagy-dependent and also-independent pathways (reviewed in[154,232])
Urolithin A	↑lifespan (W), healthspan (W), memory (W, M)	Autophagy/mitophagy induction[113,175,176]
Actinonin	↑memory (W, M)	Autophagy/mitophagy-dependent pathway[113]
Tomatidine	↑lifespan (W), healthspan (W)	Mitophagy induction via the SKN-1/Nrf2 pathway[117]
Trehalose	↑lifespan (W), healthspan (W)[233]	?
myo-inositol (MI)	↑lifespan (W), healthspan (W)	Via PINK1-dependent mitophagy[234]

Abbreviations: Yeast (Y), Worms (W), Flies (F), Mice (M), Humans (H), Rat (R), ↑ (Overexpression), ↓ (Reduced expression), NR (nicotinamide riboside), NMN (nicotinamide mononucleotide),

[1]No extension in WT mice with normal diet, but extended lifespan in the high-fat diet fed mice[94].

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