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Autophagy and the cell biology of age-related disease

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Macroautophagy (autophagy) is a conserved lysosomal degradation process essential for cellular homeostasis and adaption to stress. Accumulating evidence indicates that autophagy declines with age and that impaired autophagy predisposes individuals to age-related diseases, whereas interventions that stimulate autophagy often promote longevity. In this Review, we examine how the autophagy pathway restricts cellular damage and degeneration, and the impact of these functions towards tissue health and organismal lifespan.

Aging—the time-dependent decline in cellular and tissue function—occurs in all metazoan organisms. Although initially perceived as a progressive, entropy-driven deterioration of tissues towards non-functionality and death, we now understand that the aging process is partly controlled by genetics[1]. Evidence of this relationship emerged from screens in model organisms identifying numerous mutants that either extend lifespan or accelerate age-related decline[1]. The discovery of these conserved genetic determinants of lifespan revealed aging as a biological programme directed by specific signalling pathways and cellular machineries[1]. Evidence implicates critical roles for diverse stress response pathways in promoting longevity and healthy aging[1,2]. These pathways, including autophagy, mitigate environmental pressures and extend lifespan through biological programs that limit tissue damage and promote repair and maintenance[1]. These cytoprotective programs progressively wane during aging, ultimately compromising tissue function and survival.

Autophagy is a conserved lysosomal degradation pathway essential for cellular homeostasis and adaptation to stress[3,4]. During stress, autophagy facilitates survival through clearance of damaged molecules and mobilization of intracellular stores of energy and nutrients[4]. Abundant evidence supports autophagy as a critical regulator of lifespan[5,6]. Many physiological and pharmacological interventions that extend lifespan, such as caloric restriction (CR) and the mTOR inhibitor rapamycin, induce autophagy[5]. In contrast, autophagy declines with age and autophagy impairment predisposes to assorted age-related diseases, including neurodegeneration and arthritis[6]. Here, we review the biological pathways through which autophagy mitigates cellular damage during aging and discuss strategies to induce autophagy to combat age-related disease.

Overview of the autophagy pathway

Autophagy describes a collection of cellular self-digestion processes that deliver cytoplasmic material to the lysosome for breakdown. Three principal routes of autophagy exist—macroautophagy, microautophagy and chaperone-mediated autophagy (CMA), which mainly differ in how cytosolic material is delivered to lysosomes[3]. This Review focuses on macroautophagy, hereafter termed autophagy, which involves sequestration of cargo within double-membrane vesicles called autophagosomes that subsequently fuse with lysosomes to degrade cargo[3]. The core autophagy machinery includes numerous autophagy-related proteins (ATGs) that assemble into complexes to promote autophagosome formation and maturation[7].

Signalling pathways regulate autophagy during aging

Autophagy is induced by diverse stresses, including nutrient deprivation, growth factor withdrawal, genotoxic stress, and damaged proteins and organelles[4]. The catabolic functions of autophagy mitigate these stresses through the clearance of damaged components and the supply of molecules that sustain core metabolism[3]. Hence, stress-induced autophagy is part of a broader metabolic shift that promotes cell and organismal survival by prioritizing repair and maintenance over growth.

Among the diverse signalling networks regulating autophagy, two kinases linked to the regulation of aging and lifespan deserve special mention: mechanistic target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) (Fig. 1). mTOR, a negative regulator of autophagy, integrates signals from nutrient and growth factor pathways to control cellular growth and metabolism[8]. In nutrient-replete conditions, mTOR phosphorylates unc-51-like autophagy activating kinase (ULK)1 and sequesters it in a complex with ATG13 and FIP200 (an ULK-interacting protein), thereby inhibiting autophagy[8]. Stresses, such as nutrient starvation, inhibit mTOR and promote autophagy. In contrast, AMPK positively regulates autophagy. AMPK is activated by energy stress, which it senses through increases in the AMP/ATP ratio[8]. On activation, AMPK stimulates autophagy through multiple mechanisms. First, it phosphorylates and activates ULK1 and Beclin-1–VPS34 to promote the early steps of autophagosome induction[8]. Second, AMPK inhibits mTOR by phosphorylating and inhibiting RAPTOR (regulatory-associated protein of mTOR), an adaptor important for mTOR kinase activity[8]. Finally, AMPK simulates tuberous sclerosis complex (TSC)1–TSC2 complex activity, which inhibits mTOR[8].

Autophagy and longevity

Aging correlates with reduced autophagy in diverse organisms. Early studies revealed that aged rats, primary human cells and *Caenorhabditis elegans* have reduced lysosomal proteolysis compared to younger counterparts[9,10]. Aging is also accompanied by reduced expression of several *ATGs* in *Drosophila* and rodent tissues[11–13]. In humans, *ATG5*, *ATG7* and *BECN1* (encoding Beclin-1) are down-regulated during normal aging[14], whereas age-associated diseases such as neurodegeneration, osteoarthritis and cardiomyopathy exhibit reduced autophagy[15,16]. Recent work in *C. elegans* corroborates *bona fide* reductions in autophagic flux in vivo over organismal lifetime, evidenced by an age-dependent accumulation of immature autophagosomes and decreased autophagic degradation

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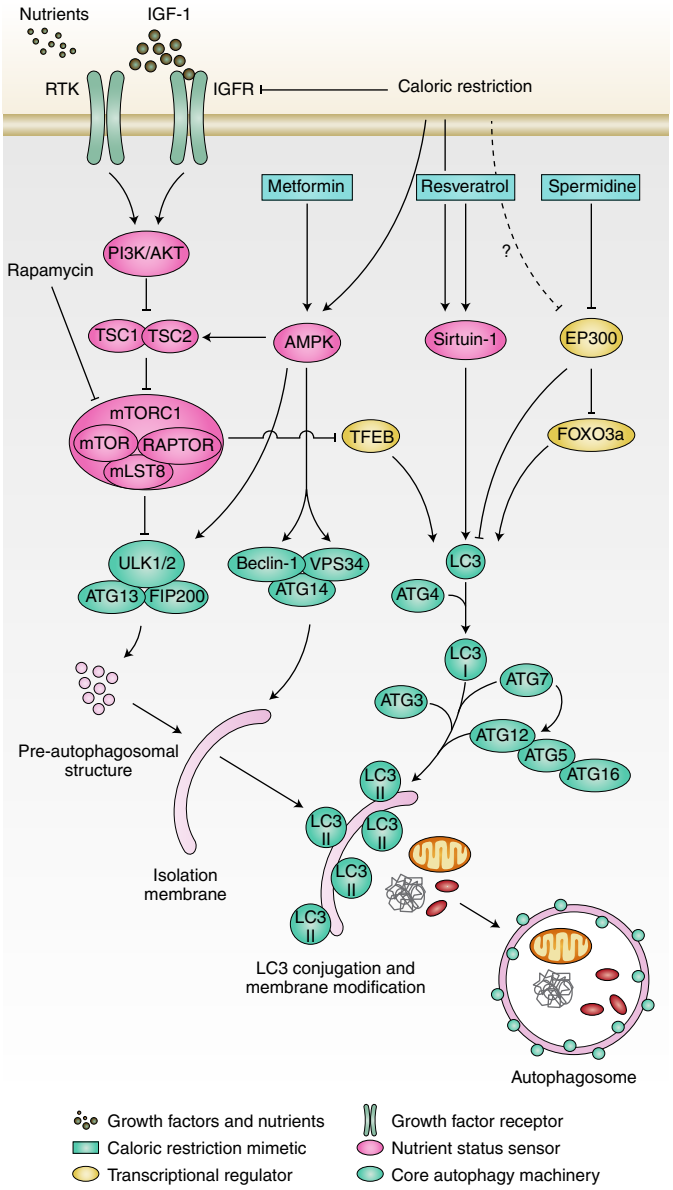

Fig. 1 | Signalling pathways that regulate autophagy and lifespan. Signals are integrated by core autophagy machinery, hierarchically controlling discrete steps within the autophagy trafficking pathway. Nucleation of the pre-autophagosomal structure (PAS), the first step in the autophagy pathway, is regulated by the ULK1/2–ATG13–FIP200 kinase complex. Nutrients and growth factors (IGF-1) trigger signalling through receptor tyrosine kinases (RTKs), such as the insulin growth factor receptor (IGFR), to downstream effectors, including PI3K/AKT and TSC1/2. Ultimately, these signals converge on mTORC1 (mTOR, RAPTOR and mLST8) and AMPK, which coordinately modify the ULK complex to affect its functions in PAS formation. Caloric restriction, rapamycin and metformin impact signalling through the mTOR–AMPK axis to activate ULK1/2 and promote early steps in the autophagy pathway. PAS is subsequently modified by a complex of Beclin-1, ATG14 and VPS34, to form the isolation membrane, expansion of which is associated with two ubiquitin-like reactions involving ATG7, ATG5, ATG12, ATG16 and ATG3, ultimately conjugating phosphatidylethanolamine (PE) to LC3. The activity of the LC3 conjugation machinery is controlled by the transcription factors TFEB and FOXO3a, which regulate expression of ATGs, and through post-transcriptional acetylation of core autophagy proteins involving the deacetylase Sirtuin-1 and acetyltransferase EP300. Caloric restriction, resveratrol and spermidine affect transcriptional and acetylation-dependent regulation of the LC3 conjugation machinery to activate autophagy. LC3–PE conjugation targets LC3 to autophagosomal membranes where it is required for membrane expansion and cargo sequestration. Finally, the autophagosome is sealed and sequestered cargo is delivered to the lysosome through autophagosome–lysosome fusion. Arrows indicate activating signals, whereas blunt-end lines represent inhibitory signals. Dashed lines with question marks indicate signals that may be inferred from experimental data, but not formally demonstrated.

across all tissues examined[17]. Furthermore, the accumulation of uncleared autophagosomes during aging may exacerbate neuronal dysfunction that contributes to age-related disease in worms[18]. The reasons for this age-dependent autophagy impairment remain unclear, but may involve reduced expression of genes important for autophagosome–lysosome fusion, such as lysosome-associated membrane protein 2 (*LAMP2A*), which decrease with age[19]. In mice, an age-dependent decrease in autophagosome numbers has recently been reported, as might be predicted by the age-related decline in *ATG* expression in humans[20].

Although the relationship between autophagy and human aging remains complex, studies in model systems support a critical role for autophagy in longevity and geroprotection. A link between autophagy and longevity emerged from *C. elegans* demonstrating

that *atg* genes are required for lifespan extension in long-lived *daf-2* mutants[21], which have impaired insulin–insulin-like growth factor-1 (IGF-1) receptor signalling (Fig. 1, Table 1). Autophagy appears to be activated in *daf-2* mutants, although early studies did not conclusively demonstrate increases in autophagic flux in *daf-2* mutant worms[21]. Nonetheless, *bec-1* (encoding Beclin-1, also known as ATG6), *atg-7, atg-12* and *lgg-1* (also known as LC3) are required for *daf-2*-mediated longevity, supporting that this phenotype is autophagy-dependent[21–23]. *daf-2* mutants also require the transcription factor *daf-16* (also known as FOXO) for enhanced longevity, which can promote the upregulation of *atg* genes in worms, flies and mammals[24–26]. *Atg7* or *Atg8* loss-of-function mutations in *Drosophila* reduce lifespan, increase sensitivity to stress and promote accumulation of ubiquitin-positive aggregates within neurons[13,24]. Because autophagy is required in mice for neonatal survival[27], tissue-specific *Atg* knockouts have been employed to study the relationship between autophagy and aging in murine models. These models reinforce that autophagy is required for healthy aging, and deficiencies promote accumulation of damaged mitochondria and protein aggregates[6]. These findings suggest impaired autophagy predisposes individuals to age-related diseases and reduced lifespan in diverse species. Recent work in *Becn1*$^{F121A/F121A}$ mutant mice, which stimulates autophagy by suppressing interaction of Beclin-1 with the negative regulator Bcl-2, demonstrates that enhanced basal autophagy increases longevity and diminishes numerous age-related phenotypes in vivo[20].

A crucial role for autophagy in promoting longevity is also observed in the context of lifespan-extending stimuli[5]. Reduced nutrient intake without malnutrition, termed CR, is a robust intervention that delays aging in nearly all species[28–30]. Importantly, autophagy inhibition diminishes the lifespan-enhancing effects of CR. The enhanced longevity of *C. elegans eat-2* mutants, which consume less food due impaired pharynx function, is attenuated on inactivation of *atg* genes or transcriptional autophagy regulators such as *hlh-30* (also known as TFEB) and *pha-4* (also known as FOXA)[31–33]. Studies in *C. elegans* examining autophagy in CR illuminate tissue-specific functions for this pathway in healthy aging and lifespan extension[31]. CR-induced autophagy likely involves multiple mechanisms that converge to inhibit mTOR signalling. This is supported by epistasis experiments in yeast and worms, demonstrating that lifespan extension by CR is not enhanced by mTOR inhibition[34,35]. mTOR inhibition using rapamycin mimics several beneficial effects of CR and extends the lifespan of diverse species[36]. Autophagy, at least in part, mediates these phenotypes because mTOR inhibition does not extend the lifespan of *ATG*-deficient invertebrates[34,37,38]. Rapamycin also induces autophagy in mammals, where it extends lifespan and promotes tissue-specific proteostasis[39,40]. These observations broach the utility of mTOR inhibitors as autophagy-inducing therapies for age-related diseases[41]. Nevertheless, pathways other than autophagy likely contribute to the lifespan-extending benefits of mTOR inhibition; rapamycin possesses potent immunosuppressive properties and also reduces protein translation, which may extend lifespan by restraining inflammation and alleviating protein imbalances, respectively, that contribute to aging[42,43]. Other *C. elegans* longevity paradigms requiring autophagy include mutant *glp-1* ablation of the germline[44], AMPK overexpression[45], hormetic responses to heat stress[46], impaired mitochondrial respiration[47] and Sirtuin-mediated lifespan extension[48]. However, the role of autophagy in Sirtuin longevity paradigms remains controversial[5]. Considering the pleiotropic effects of longevity pathways in model organisms, further studies are needed to ascertain to what extent autophagy contributes to the beneficial effects of lifespan-extending stimuli in humans.

Autophagy, proteostasis and neurodegeneration

Autophagy is critical for maintaining protein homeostasis (proteostasis), particularly within post-mitotic cells that are not protected by the dilutive effects of cell division[49] (Fig. 2). Autophagy cooperates with the ubiquitin proteasome system (UPS) to degrade toxic proteins[49]. During aging, post-mitotic cells exhibit impaired proteostasis, which correlates with the functional decline of protein quality control mechanisms, including autophagy[49]. Here, we focus on how such declines contribute to age-related neurodegenerative diseases (Table 1).

In the brain, impaired autophagy in neurons promotes the accumulation of toxic protein aggregates and damaged organelles linked to neurodegenerative disorders[16]. Neuron-specific knockout of *Atg5*, *Atg7*, *Rb1cc1*/*FIP200* or *Ulk1/2* in mice results in the accumulation of ubiquitin-positive aggregates, leading to progressive neurodegeneration and premature death[50–54]. Similarly, *Atg5* or *Atg7* deletion in Purkinje neurons induces the build-up of ubiquitinated protein aggregates and cell autonomous degeneration[55,56]. The neuronal accumulation of aggregates in otherwise normal mice supports a critical role for autophagy in clearing aggregate-prone proteins. *ATG5, ATG7* and *BECN1* expression in the human brain decreases with age, possibly contributing to reduced autophagy and neurodegeneration observed in advanced age[14].

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder associated with pathogenic protein aggregation[16]. AD involves the deposition of extracellular β-amyloid (Aβ) plaques and intracellular neurofibrillary tangles containing hyperphosphorylated tau[16]. Aβ and tau are substrates for autophagy and the abundance of autophagic structures filled with undigested substrates suggests autophagy is impaired in AD (refs [57,58]). Studies implicate defective autophagosome maturation in the development of Aβ and tau aggregates, which may be exacerbated by the age-related decline in neuronal autophagy[59,60]. AD-associated mutations in the transmembrane protein presenilin-1, which cause a form of familial early-onset AD, lead to impaired autophagic flux through faulty lysosomal targeting of the v-ATPaseV_0a1 subunit and the resultant defects in lysosomal acidification[59]. Studies in CA1-hippocampal neurons from sporadic AD patients have revealed impairment of autophagic flux in late-stage disease, despite robust autophagosome biogenesis[60]. This contrasts with neurons from early-stage AD patients that efficiently clear autophagic substrates and retain lysosomal function[60].

Genome wide association studies (GWAS) have implicated the phosphatidylinositol-binding clathrin assembly protein (*PICALM*) gene locus in AD (ref. [61]). *PICALM* encodes a clathrin adaptor required for the endocytic uptake of soluble NSF attachment protein receptors (SNAREs), including VAMP8 (ref. [62]). Genetic modulation of PICALM levels perturbs autophagic flux, promoting tau accumulation and proteotoxicity in *Drosophila* and zebrafish models, respectively[63]. Perturbation of axonal transport can also impair lysosomal function, potentially promoting amyloid plaque pathology[64]. Nevertheless, whether these pathways are affected in sporadic AD or aging remains unclear.

Although AD pathogenesis is predominately associated with impaired autophagosome maturation, impaired autophagosome formation may also contribute[65]. *BECN1* is decreased in the brains of AD patients and, in a murine model of AD, Beclin-1 haploinsufficiency reduces autophagy and promotes age-dependent accumulation of Aβ (ref. [65]). Accordingly, enforcing Beclin-1 expression mitigates amyloid pathology[65]. The importance of basal autophagy in controlling Aβ production and AD pathogenesis is supported by studies using *Becn1*$^{F121A/F121A}$ knock-in mice. When intercrossed with mouse models of AD, the resultant *Becn1*$^{F121A/F121A}$ offspring with hyperactive autophagy show reduced amyloid accumulation, delayed cognitive decline and improved survival[66].

Despite considerable evidence highlighting the cytoprotective roles for autophagy in AD, its functions in pathogenesis may be more nuanced. A significant proportion of Aβ may be generated in autophagosomes containing amyloid precursor protein (APP) and γ-secretase, the enzyme that mediates APP cleavage to Aβ (ref. [67]). Autophagy may also facilitate Aβ secretion; deletion of *Atg7* in mice constitutively expressing APP exhibit reduced extracellular Aβ and

Table 1 | Evidence from model organisms that support roles for autophagy in the regulation of aging and age-related disease

Organism	Manipulation	Aging phenotype	Role of autophagy in aging phenotype	Refs
Sacchromyces cerevisiae	Caloric restriction	Lifespan extension (CLE and RLE)	Genetic deletion of *atg-1*, *atg-7* or *atg-16* abolishes lifespan extension	5,38
	Spermidine	Lifespan extension (CLE)	Genetic knockout of *atg-7* abolishes lifespan extension	144
C. elegans	Caloric restriction *eat-2* mutant (impaired pharynx function)	Lifespan extension	Loss-of-longevity phenotype on *bec-1, atg7, atg-18* RNAi or intestine-specific *atg-18*, *lgg-1/lgg-2/ATG8* RNAi	23,31,32
	Resveratrol treatment	Lifespan extension	Loss-of-longevity phenotype on *bec-1* RNAi	48
	Spermidine treatment	Lifespan extension	Loss-of-longevity phenotype on *bec-1* RNAi	144
	atp-3 RNAi (impaired mitochondrial respiration)	Lifespan extension	Loss-of-function mutations in *unc-51, bec-1* or *atg-18* abolish lifespan extension	47
	daf-2 (insulin/IGF-1 receptor) loss-of-function mutant	Lifespan extension	Loss-of-function mutations in *bec-1*, or on *lgg-1/LC3, atg-7* or *atg-12* RNAi, abolishes lifespan extension	21
	daf-15 mutant (Raptor)	Lifespan extension	Loss-of-longevity phenotype on *bec-1* RNAi	23
	glp-1 loss-of-function mutant (germline deficient)	Lifespan extension	Loss-of-longevity phenotype on *bec-1, lgg-1/LC3, atg-18* RNAi	44
	Hormetic heat stress	Lifespan extension	Loss-of-longevity phenotype on *bec-1, lgg-1/LC3, unc-51/ULK1* RNAi	46
	TOR/let-363 RNAi	Lifespan extension	Loss-of-longevity phenotype on *bec-1* RNAi	23
	Transgenic expression of *sir-2*	Lifespan extension	Loss-of-longevity phenotype on *bec-1* RNAi	48
Drosophila melanogaster	Rapamycin treatment	Lifespan extension	Loss-of-longevity phenotype on *Atg5* RNAi	37
	Spermidine treatment	Lifespan extension	Genetic deletion of *Atg7* abolishes lifespan extension	144
	Brain-specific transgenic over-expression of *Atg8a*	Lifespan extension	Improved neuronal proteostasis and resistance to oxidative stress	13
	Neuronal transgenic overexpression of Atg1/ULK1	Lifespan extension	Enhanced autophagy in neurons and systemically, including muscle and intestine; and altered insulin-like signalling	45
	Neuronal transgenic overexpression of AMPKα1	Lifespan extension	Enhanced autophagy in neurons and systemically, including muscle and intestine; and altered insulin-like signalling	45
	Muscle-specific transgenic over-expression of *dFOXO*	Lifespan extension	Improved muscle proteostasis	11
Mice	Caloric restriction	Lifespan extension	Enhanced autophagy and delayed age-related changes in diverse tissues	30
	Metformin treatment	Lifespan extension	Enhanced autophagy, improved cardiac function, insulin sensitivity and glucose metabolism, and reduced inflammation and hepatic steatosis	140 a
	Rapamycin treatment	Lifespan extension	Enhanced autophagy and delayed age-related changes in diverse tissues	40
	Resveratrol treatment of mice on HFD	Increased healthspan and lifespan	Improved mitochondrial homeostasis, increased insulin sensitivity, reduced hepatic inflammation and steatosis	149
	Spermidine treatment	Increased healthspan and lifespan	Enhanced cardiac mitophagy and mitochondrial respiration, reduced cardiac hypertrophy and contractile dysfunction within aged mice, and cardiomyocyte-specific deletion of *Atg5* abolishes cardioprotection	150
	Atg5 transgenic overexpression (systemic)	Lifespan extension	Enhanced autophagy, increased insulin sensitivity and resistance to oxidative stress	138 a
	Becn1$^{F121A/F121A}$ knock-in (systemic)	Increased healthspan and lifespan	Enhanced autophagy in diverse tissues and reduced age-related genotoxic stress within kidney and heart, decreased renal and cardiac fibrosis, and decreased amyloid accumulation and cognitive decline in Alzheimer's disease models	20,66
	Becn1 monoallelic deletion (systemic)	Reduced healthspan	Increased susceptibility to Alzheimer's disease, incidence of spontaneous malignancies, steatohepatitis, severity of Desmin-related cardiomyopathy and renal fibrosis following basal renal collagen accumulation; reduced exercise endurance and exercise-induced insulin sensitivity	65,110,138 a

Continued

Table 1 | Evidence from model organisms that support roles for autophagy in the regulation of aging and age-related disease

Organism	Manipulation	Aging phenotype	Role of autophagy in aging phenotype	Refs
	Map1lc3b homozygous deletion (systemic)	Reduced healthspan	Increased renal fibrosis following ureteral obstruction and susceptibility to hypoxia-induced pulmonary hypertension	65,110,138 [a]
	Adipose-specific deletion of *Atg7*	Increased healthspan?	Impaired beige to white adipose transdifferentiation, protection against diet-induced obesity and insulin resistance	117
	Cardiac-specific deletion of *Atg5* (inducible)	Reduced healthspan	Accumulation of dysfunctional mitochondria, ubiquitinated proteins, cardiac hypertrophy, sarcomere disorganization, contractile dysfunction and fibrosis	138 [a]
	Chondrocyte-specific deletion of *Atg5*	Reduced healthspan	Cartilage loss, reduced proteoglycan deposition and increased susceptibility to osteoarthritis	130
	Eye lens cell-specific deletion of *Atg5*	Reduced healthspan	Accumulation of oxidized, polyubquitinated and aggregated proteins; and increased susceptibility to age-related cataracts	138 [a]
	Hepatocyte-specific deletion of *Atg5*, *Atg7* or mosaic system deletion of *Atg5*	Reduced healthspan	Increased hepatocyte mitochondrial swelling, senescence, hepatic inflammation, steatosis, fibrosis, adenomas, blood glucose imbalance and impaired liver regenerative capacity	138 [a]
	Neuronal knockout of *Atg5*, *Atg7* or *Rb1cc1*	Reduced healthspan and lifespan	Proteostasis imbalance with accumulation of protein aggregates, neuronal loss and cell death, and neurodegeneration	50,52,54
	Macrophage-specific deletion of *Atg5*	Reduced healthspan	Increased inflammasome activation and accelerated progression of atherosclerosis	138 [a]
	Pancreatic β-cell-specific deletion of *Atg7*	Reduced healthspan	Accumulation of ubiquitinated proteins, abnormal mitochondria, and distended ER, increased pancreatic β -cell death and incidence of diabetes	138 [a]
	Podocyte-specific deletion of *Atg5*	Reduced healthspan	Accumulation of oxidized and ubiquitinated proteins, ER stress within podocytes, proteinuria, podocyte loss and glomerulosclerosis	138 [a]
	Purkinje-cell-specific deletion of *Atg7*	Reduced healthspan	Purkinje cell axonal degeneration	55
	Renal tubular cell-specific deletion of *Atg5*	Reduced healthspan	Accumulation of damaged mitochondria, sensitivity to ischemic injury, tubular cell loss and impaired kidney function	133
	Skeletal muscle-specific deletion of *Atg7*	Reduced healthspan	Accumulation of abnormal mitochondria, muscle atrophy and age-dependent decrease in strength, sarcoplasmic reticulum distension and sarcomere disorganization	97

[a]Primary references cited within review. CLE, chronological lifespan extension; RLE, replicative lifespan extension; HFD, High-fat diet; ER, endoplasmic reticulum; RNAi, RNA interference.

emerging contributions of autophagy to AD pathogenesis.

Parkinson's disease (PD) is a common age-related neurodegenerative disease caused by the degeneration of dopaminergic neurons[69]. This decline is associated with increased intracellular protein aggregates containing α-synuclein (termed Lewy bodies) and other cellular perturbations, including mitochondrial dysfunction and deregulated autophagy[69]. Increased cellular levels of wild-type α -synuclein contribute to aggregation in sporadic PD; however, aggregation-prone α-synuclein mutants have also been identified in rare heritable forms of the disease[69–71]. Although α-synuclein can be degraded by macroautophagy, age-related declines in other protein quality control mechanisms, such as CMA and UPS, contribute to increased levels of α-synuclein and predispose individuals to the formation of PD-associated aggregates[72–74]. Intriguingly, following progressive accumulation of aggregates within PD dopaminergic neurons due to impairments in one quality control pathway, other clearance mechanisms diminish. Whereas α-synuclein aggregates inhibit CMA (ref. [74]), they also impair autophagosome maturation and lysosomal degradation[75]. This may partly relate to the effects of aggregates on the CMA lysosomal transmembrane receptor LAMP2A, which has ancillary functions in autophagosome result in the mislocalization of ATG9, and suppress autophagosome formation[76]. Interestingly, mislocalization of ATG9 also occurs in hereditary forms of PD caused by the *VPS35*^{D620N} dominant mutant allele[77]. *VPS35* encodes a subunit of the membrane protein–recycling retromer complex, which facilitates retrograde transport from endosomes to the trans-Golgi network[77]. The PD-associated *VPS35*^{D620N} mutant impairs these functions, leading to ATG9 mis-sorting and impaired autophagosome formation[77]. These results highlight how specific defects in the autophagy pathway, along with progressive age-related reductions in autophagy, can become amplified to impair proteostasis during neurodegeneration.

Mitophagy and age-related mitochondrial dysfunction

Dysfunctional mitochondria accumulate with age, and 'old' cells exhibit concomitant reductions in respiratory chain efficacy and ATP production as well as increased reactive oxygen species (ROS) production[78]. Although increased free radical production was initially proposed to link dysfunctional mitochondria to age-associated cellular decline, evidence from model systems paradoxically suggests that ROS prolongs lifespan, perhaps by activating hormetic responses[79]. Instead, dysfunctional mitochondria have been linked

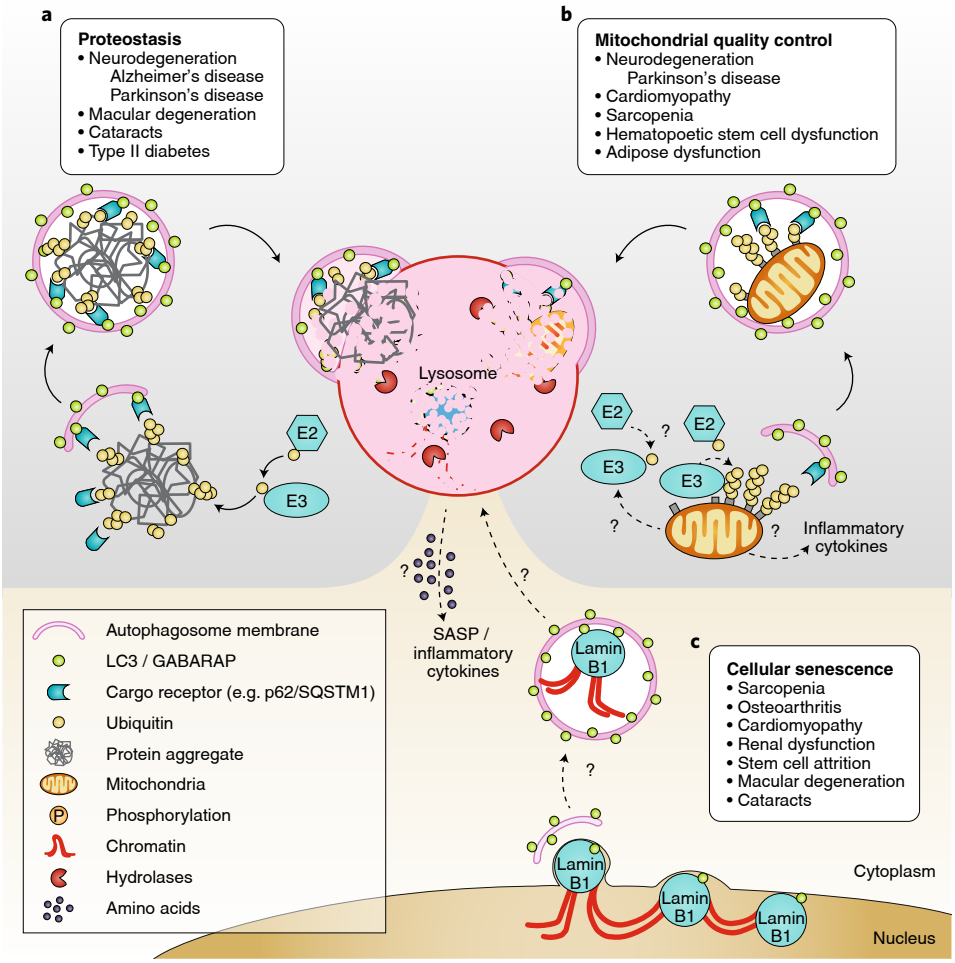

Fig. 2 | Geroprotective mechanisms of autophagy and age-related diseases manifest by autophagy dysfunction. a, Autophagy maintains proteostasis by promoting the degradation of misfolded and aggregated proteins. E2 activating enzymes and E3 ligases of the ubiquitin conjugation system promote the ubiquitination of protein aggregates (which are recognized by autophagy cargo receptors), sequestered within autophagosomes and subsequently degraded within lysosomes. **b**, Mitochondrial quality control involves the clearance of damaged mitochondria by mitophagy. E3 ubiquitin ligases are recruited to damaged mitochondria, where they promote the ubiquitination of mitochondrial proteins. Autophagy cargo receptors recognize ubiquitin-modified mitochondria, facilitating their sequestration within autophagosomes and lysosome-mediated breakdown. Mitochondrial damage-associated molecular patterns may also trigger inflammatory cytokine production. Dashed arrows with question marks highlight the largely undefined molecular mechanisms that regulate mitophagy in vivo. **c**, Autophagy regulates senescence by promoting the disassembly and degradation of the nuclear lamina. Age-related genotoxic stress stimulates LC3 and lamin B1 within the nuclear lamina to bud into the cytoplasm along with fragments of chromatin. Cytoplasmic chromatin fragments stimulate innate immune pathways, and are captured (along with LC3 and lamin B1) by autophagic membranes and delivered to the lysosome for degradation. Free amino acids released from the lysosome during senescence support anabolic activities, including the production of inflammatory cytokines that make up the SASP. Dashed arrows with question marks indicate recently discovered mechanisms by which autophagy regulates senescence, and also highlight the emerging relationship between these two pathways as an exciting area of future investigation.

to diverse cell biological disruptions associated with age-related diseases, including proteotoxicity[49], inflammasome activation[80] and senescence (termed MiDAS)[81].

Autophagy has a well-established role in clearing dysfunctional mitochondria, termed mitophagy[78] (Fig. 2). The accumulation of abnormal mitochondria within dopaminergic neurons represents another hallmark of PD and evidence suggests that defective mitophagy contributes to mitochondrial dysfunction in PD (ref. [69]). Initial insight into this pathway came from studies of PINK1 and Parkin, two mitophagy regulators genetically implicated in autosomal recessive forms of early-onset PD[82,83]. Early in vitro studies revealed that PINK1, a mitochondrial protein kinase, and Parkin, an E3 ubiquitin ligase, operate sequentially to ubiquitinylate depolarised mitochondria and enable specific engulfment by autophagosomes[84,85]. In cell culture models, PD-associated mutations in *PINK1* or *PRKN* (encoding Parkin) compromised mitophagy through diverse mechanisms, including impaired targeting of Parkin to mitochondria, disruption of Parkin E3 ligase activity and formation of aberrant intracellular protein aggregates[86–88]. However, *Pink1* and *Prkn* knockout mice fail to develop PD-associated phenotypes[89,90], and evidence from *Drosophila* and mice demonstrates Pink1/Parkin is dispensable for mitophagy in vivo[91,92], indicating factors other

than Pink1/Parkin regulate PD and mitophagy. The demonstration of prohibitin-2 as a mitophagy regulator broaches alternative quality control mechanisms that may impact neurodegeneration[93]. Additionally, PINK1 and Parkin have been implicated in suppressing inflammatory responses triggered by mitochondrial damage-associated molecular patterns, which may contribute to the loss of dopaminergic neurons in PD (ref. [94]). These studies highlight the complex role of autophagy in mitochondrial homeostasis and illustrate the need for further work to unravel how mitophagy pathways function to prevent age-related neurodegeneration.

Mitophagy is also essential for muscle health and its impairment exacerbates the development of age-related myopathies, such as sarcopenia[78] (Fig. 2). In *Drosophila*, *Pink1* or *Prkn* deficiency leads to the accumulation dysfunctional mitochondria within muscle tissues and progressive loss of indirect flight muscles[90,95]. Studies in mice corroborate this conserved role for autophagy in preserving myocyte function. Muscle-specific ablation of *Atg5* or *Atg7* results in abnormal mitochondria, oxidative stress and progressive muscle atrophy[96,97]. Similarly, mitochondrial dysfunction caused by complete knockout of *Pink1* is sufficient to induce elevated ROS levels within cardiac myocytes, predisposing to heart dysfunction and failure[98].

The incidence of heart disease dramatically increases with age[99]. Cardiac aging is characterized by hypertrophy, fibrosis, and accumulation of misfolded proteins and dysfunctional mitochondria[99]. In mice, autophagy declines with age in the heart and cardiac-specific *Atg5* deletion results in severe cardiomyopathy with systolic dysfunction during aging, accompanied by sarcomeric disorganization and the accumulation of dysfunctional mitochondria[100]. The important anti-aging functions of autophagy in cardiac tissue, including maintenance of mitochondrial homeostasis, are reinforced by studies in *Becn1*$^{F121A/F121A}$ autophagy gain-of-function mice, which show decreased age-related cardiomyocyte genotoxic stress and reduced cardiac hypertrophy and fibrosis[20]. These data suggest that restoring autophagy in the aging heart may preserve mitochondrial integrity and prevent cardiovascular disease.

Sarcopenia, the age-related degenerative loss of skeletal muscle mass and strength, is a principal cause of functional decline and frailty within older adults[101]. The accumulation of dysfunctional mitochondria contributes to sarcopenia and is partially associated with impaired autophagic function in aged skeletal muscle[101]. Expression of several *ATG* genes decreases with age in muscle tissue[102–104]. Muscle-specific *Atg7* knockout mice display accelerated aging characterized by oxidative stress, mitochondrial dysfunction, muscle loss and degeneration of neuromuscular junctions[104]. This phenotype is similar to that observed following sustained activation of mTOR complex 1 (mTORC1) or loss of *Prkaa1* (encoding AMPKα1) in skeletal muscle, which suppress autophagy[105,106]. AMPK activity decreases in aged human skeletal muscle, intimating a role for impaired mitophagy in progressive muscle decline with age[107].

Collectively, these findings suggest that interventions to enhance autophagy may delay or reverse sarcopenia during aging. Regular physical exercise and nutritional interventions can slow age-related muscle decline and these beneficial effects are, in part, mediated by autophagy[108]. Physical exercise induces autophagy and enhances mitochondrial function in skeletal muscle, and the ability of strength training to preserve muscle mass during aging is associated with increased autophagy[108–110]. Importantly, these effects require autophagy, as exercise-trained *Becn1*$^{+}$ mice show negligible improvements in mitochondrial content in skeletal muscle and endurance capacity[108,110]. In addition, CR prevents the age-related decline in muscle mass and strength[111,112]. Rodents subject to lifelong CR demonstrated higher expression of *Atgs* within skeletal muscle relative to controls, and this correlates with reduced oxidative damage and apoptosis over organismal lifetime[113]. A study in humans corroborates these results, reinforcing that nutritional regimens resulting in activated autophagy preserve muscle health through the removal of dysfunctional mitochondria and proteins[112]. Despite these promising results with CR regimens, it remains unclear whether pharmacological autophagy inducers can similarly preserve muscle health or suppress sarcopenia.

Mitochondrial dysfunction and deregulated autophagy also perturb adipose tissue during aging[114] (Table 1). Adipogenesis declines with age, which is linked to inflammatory signals originating from other tissues in the body[114]. These signals are produced by cells that have undergone MiDAS, a form of proliferative arrest induced in response to mitochondrial damage[81]. Although the status of autophagy in MiDAS remains unknown, evidence suggests impaired mitophagy within senescent cells contributes to the production of inflammatory cytokines that induce senescence through paracrine mechanisms[81,115]. The inflammatory signals from MiDAS can suppress the differentiation of preadipocytes, and the elimination of senescent cells restores adipogenesis in aged mice[81,116]. One can speculate that senescence-associated inflammatory signals control adipogenesis by modulating autophagy, given that genetic or pharmacological inhibition of autophagy in preadipocytes also prevents their differentiation[117]. These studies illustrate how impaired mitophagy functions as a driver of age-related degeneration.

Autophagy, senescence and stem cells

Autophagy regulates cellular senescence, the process of stable proliferative arrest of mitotic cells triggered by diverse stresses, including telomere attrition, DNA damage, mitochondrial dysfunction and aberrant hyperproliferative stimuli[118]. Although proliferative barriers imposed by senescence temporarily protect against the harmful effects of damaged cells, the accrual of senescent cells within aged tissues contributes to aging and age-related diseases[118]. Senescence primarily affects aging in two ways. First, senescent cells elaborate proinflammatory cytokines that impact tissue homeostasis; second, senescence of stem cells impairs tissue regeneration[118]. The development of transgenic mice enabling selective elimination of senescent cells has been instrumental for assessing the contributions of senescence to age-related disease. These models reveal that many tissues, including muscle, eye and adipose tissue, accumulate p16/INK4a-positive senescent cells with age, which triggers age-related diseases, such as sarcopenia, cataracts and adipose dysfunction[119,120].

Cell culture models of oncogene-induced senescence illuminated the initial links between autophagy and senescence, revealing that autophagy facilitates the production of inflammatory molecules that reinforce senescence through autocrine pathways, termed the senescence-associated secretory phenotype (SASP)[121]. Autophagy also promotes senescence through degradation of the nuclear lamina and chromatin[122] (Fig. 2). Senescent cells harbouring DNA-damage bud-off portions of their nucleus in an LC3-dependent manner, generating cytoplasmic chromatin fragments that are then degraded by autophagy[122]. This unique process may be critical for the SASP and immune clearance of DNA-damaged cells by triggering cyclic GMP-AMP synthase (cGAS) and stimulator of interferon genes (STING)[123–125]. However, the contributions of autophagy to senescence remain complex, as autophagy inhibition can promote senescence in certain contexts through the transcription factor, GATA4 (ref. [126]).

Autophagy also contributes to osteoarthritis, an age-related degenerative joint disease characterized by inflammation and loss of joint cartilage[15] (Table 1). Senescent cells accumulate in osteoarthritic joints with age; this correlates with a decline in ATG expression[127,128] and may involve dysregulated signalling through the mTOR (ref. [129]). Chondrocyte-specific deletion of *Atg5* predisposes individuals to age-related osteoarthritis with loss of proteoglycan deposition[129,130]. Explant cultures of human joint tissue containing senescent chondrocytes exhibit similar cartilage deposition defects, which can be rescued by targeting senescent cells[128]. These findings suggest that loss of autophagy promotes senescence of chondrocytes, driving the decline of aged joint cartilage.

Table 2 | Select compounds that induce autophagy

Agent	Developmental status	Mechanism of autophagy induction
ABT-199 (also known as Venetoclax)	Approved for the treatment of chronic lymphocytic leukaemia (CLL)	BH3 mimetic and Beclin-1 activator
ABT-263 (also known as Navitoclax)	Phase I/II clinical trials for cancer	BH3 mimetic and Beclin-1 activator
ABT-737	In preclinical development	BH3 mimetic and Beclin-1 activator
Alvespimycin (also known as 17-DMAG)	Discontinued from clinical tests (hepatotoxicity)	HSP90 inhibitor and inhibition of Akt/mTOR/p70S6K signalling?
Beclin-1-derived peptide	In preclinical development	Beclin-1 activator
Carbamazepine	Approved for treatment of seizures and bipolar disorders	Reduction in Ins(1,4,5)P_3 and inositol levels
Clonidine and Rilmenidine	Approved for the treatment of hypertension	Reduction in cAMP levels
Caloric restriction	Not available	Multiple
Everolimus (also known as RAD001)	Approved for cancer therapy	Inhibition of mTORC1
Geldanamycin	Discontinued from clinical tests (hepatotoxicity)	Inhibition of Akt/mTOR/p70S6K signalling?
Hydroxycitrate	Nutritional supplement	CRM and AMPK activation
Lithium	Approved for treatment of bipolar disorders	Reduction in Ins(1,4,5)P_3 and inositol levels
Metformin	Approved for type II diabetes	CRM and AMPK activation
Perhexiline	Approved for angina	CRM, AMPK activation and Acetyl-CoA reduction
Physical exercise	Not available	Multiple
Rapamycin (also known as sirolimus)	Approved for immunosuppression and cancer therapy	Inhibition of mTORC1
Resveratrol	Nutritional supplement	CRM and SIRT1 activation
Statins	Approved for obesity	Depletion of geranylgeranyl disphosphate, AMPK activation and mTORC1 inhibition
Spermidine	Nutritional supplement	CRM and EP300 deacetylase inhibitor
Tanespimycin (also known as 17-AAG)	Discontinued from clinical tests	HSP90 inhibitor and inhibition of Akt/mTOR/p70S6K signalling?
Temsirolimus (also known as CCI-779)	Approved for cancer therapy	Inhibition of mTORC1
Torins	Experimental agent	Inhibition of mTORC1
Trehalose	Nutritional supplement, Phase I/II clinical trials for bipolar disorder and vascular aging	Glucose transporter inhibition and AMPK activation
Trifluoperazine	Approved for schizophrenia	Dopamine agonist and unknown

Nevertheless, cellular senescence may exert beneficial effects that delay disease. Senescent cells accumulate in the kidney, renal tubule and interstitium during the aging process and in response to acute injury[131]. Although the elimination of senescent cells from the kidney attenuated aging glomerulosclerosis[120], mice with proximal tubules lacking *Atg5* demonstrate impaired senescence associated with elevated renal cell death[132,133]. The geroprotective functions of autophagy in the kidney are further supported by studies in *Becn1*$^{F121A/F120A}$ mice, which showed reductions in age-related renal damage and fibrosis[20].

The progressive decline of stem cells limits tissue regenerative responses, a major driver of the aging process[134]. Studies implicate autophagy in stem cell protection from metabolic stress and age-related impairment of autophagy contributes to progressive loss of tissue stem cell activity[134]. Autophagy supports the maintenance of muscle stem cells, called satellite cells[135]. Aging impedes satellite cell autophagy, which correlates with the accumulation of dysfunctional mitochondria, senescence and impaired regenerative capacity[135]. Although the mechanisms contributing to impaired autophagy in aged satellite cells remain unclear, rapamycin or *Atg7* overexpression restores the function of geriatric satellite cells and promotes new muscle fibre formation[135]. Conversely, *Atg7* deletion leads to satellite cell loss, with the remaining cells phenotypically resembling those in aged mice[135]. Thus, autophagy is required to prevent muscle satellite cell senescence and its progressive failure with age abrogates regenerative capacity and promotes sarcopenia.

Hematopoietic stem cells (HSCs) also require autophagy to sustain regenerative capacity; roughly 30% of aged HSCs exhibit high levels of autophagy, maintaining a low metabolic state and long-term regeneration potential similar to young HSCs[136,137]. However, the remaining 'old' HSCs develop defects in autophagy, which impairs self-renewal and regenerative functions[137]. *Atg5* or *Atg12* genetic ablation in 'young' murine HSCs causes the accumulation of mitochondria and an activated metabolic state, thereby impairing blood system regeneration[137]. Accordingly, ATG-deficient mice exhibit premature blood aging, including increased cellularity in the peripheral blood, and skewed ratios of circulating myeloid cells, a phenotype reminiscent of myeloid-bias observed in geriatric mice[137] (Table 1).

Autophagy modulators to treat age-related disease

The links between impaired autophagy and aging have spurred interest in developing agents that enhance autophagic function for the treatment of age-related diseases. Broadly, these strategies focus on inducing autophagy either through agents that mimic the biochemical and physiological effects of CR, so-called CR mimetics (CRMs), or compounds that stimulate autophagy through alternative pathways[138].

CRMs activate autophagy by targeting nutrient-sensing pathways, including mTOR, AMPK, IGF-1 and Sirtuins[139]. Although the allosteric mTOR inhibitor rapamycin extends mammalian lifespan and ameliorates neurodegeneration and osteoarthritis in mice[40,42,127], side-effects (such as immunosuppression and insulin resistance) have limited its broad application as an anti-aging therapy[42]. This has fuelled the development of mTOR inhibitors with superior pharmacodynamics as well as alternative CRMs, such as metformin, resveratrol and spermidine.

The biguanide metformin, currently approved to treat diabetes, may also act as a CRM that delays aging[140]. In addition to affecting glucose metabolism, metformin inhibits mitochondrial respiration, mTOR and IGF-1 signalling, and activates AMPK, thereby inducing autophagy[140] (Fig. 1). Although the mechanism remains unclear, metformin extends lifespan, delays neurodegeneration in mice and reduces the risk of dementia in humans[140]. These findings have prompted the TAME (Targeting Aging with Metformin) clinical trial to specifically examine the effects of this drug on several age-related diseases, potentially paving the way for metformin to become an FDA-approved anti-aging therapy[140].

Resveratrol and spermidine are nutritional supplements that may act as geroprotective CRMs (ref. [139]). Both agents extend the lifespan of diverse organisms and ameliorate neurodegeneration, metabolic dysfunction and inflammation in mice[139,141] (Table 1). CR, spermidine and resveratrol cause deacetylation of numerous proteins, including molecules regulating autophagy[139], prompting the hypothesis that CR and CRMs induce autophagy through protein deacetylation. Although resveratrol and spermidine target autophagy using the acetylproteome, they likely act through divergent mechanisms. Resveratrol stimulates autophagy by SIRT1-mediated deacetylation[48,141], whereas spermidine inhibits the acetyltransferase EP300, which acetylates and inhibits several autophagy proteins[142,143] (Fig. 1). Nevertheless, the geroprotective functions of resveratrol and spermidine require autophagy because their capacity to extend lifespan is abolished following knockdown of essential *ATG*s (ref. [48,144]).

In addition to CRMs and well-established autophagy inducers (Table 2), perhaps the most exciting class of anti-aging therapeutics to emerge are agents that eliminate senescent cells, termed 'senolytic' drugs—most notably, BH3-mimetics that inhibit anti-apoptotic Bcl-2 family members up-regulated within senescent cells[145]. In addition to promoting apoptosis in senescent cells, BH3-mimetics, such as Navitoclax/ABT-263 and ABT-737, also induce autophagy by relieving Bcl-2 inhibition of Beclin-1 (ref. [138]). Nevertheless, how autophagy directs senescent cell responses to senolytic therapies remains unclear. If autophagy is cytoprotective against therapy-induced senolysis, one can speculate that autophagy inhibition enhances senolytic activity. However, given that autophagy can potentiate cell death in certain contexts[146,147], autophagy may enhance senolytic activity. In support, a small molecule screen identified 15 autophagy-inducing compounds that selectively targeted senescent cells, including multiple heat shock protein 90 (HSP90) inhibitors that suppressed geriatric maladies in progeroid mice[148]. These findings support employing pharmacological autophagy inducers to treat age-related disease.

Concluding perspectives

Loss of autophagy creates diverse cellular dysfunctions that exacerbate the aging process, whereas enhanced autophagy generally promotes cellular homeostasis and function to prolong lifespan and improve healthspan. Because reduced autophagy is implicated in numerous age-related diseases, such as neurodegeneration, sarcopenia and osteoarthritis, the therapeutic upregulation of autophagy offers potential for treating age-related disorders. Continuing to delineate the cell biological functions of autophagy in afflicted and healthy tissues during aging is crucial in tailoring such therapies to effectively treat age-related disease.

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Competing interests

J.D. serves on the Scientific Advisory Board for Vescor Therapeutics, LLC. B.L. is a Scientific Founder of Casma Therapeutics, Inc.

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