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Review

Autophagy in ageing and ageing-associated diseases

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Autophagy is a cell self-digestion process via lysosomes that clears "cellular waste", including aberrantly modified proteins or protein aggregates and damaged organelles. Therefore, autophagy is considered a protein and organelle quality control mechanism that maintains normal cellular homeostasis. Dysfunctional autophagy has been observed in ageing tissues and several ageing-associated diseases. Lifespan of model organisms such as yeast, worms, flies, and mice can be extended through promoting autophagy, either by genetic manipulations such as over-expression of *Sirtuin 1*, or by administrations of rapamycin, resveratrol or spermidine. The evidence supports that autophagy may play an important role in delaying ageing or extending lifespan. In this review, we summarize the current knowledge about autophagy and its regulation, outline recent developments *ie* the genetic and pharmacological manipulations of autophagy that affects the lifespan, and discuss the role of autophagy in the ageing-related diseases.

Keywords: autophagy; ageing; ageing-associated diseases; cancer; neurodegenerative diseases; Sirtuin 1; p53; rapamycin; resveratrol; spermidine

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Introduction

Ageing is a complex process in which continuous accumulations of damages to molecules, organelles, cells, tissues and organs lead to a progressive decline in function and rise in vulnerability to diseases and eventually to organism death. Autophagy is a catabolic process involving the degradation of cellular components through the lysosomal machinery. It is a tightly regulated process that plays a role in cell growth, development, and homeostasis that maintain a balance between the synthesis, degradation, and subsequent recycling of cellular products.

The hypothesis that autophagy is involved in delaying ageing and extending lifespan is partially supported by three lines of evidence: First, the abundance of autophagy related proteins and autophagic activity decline over ageing. One of the common characteristics of senescent cells is the accumulation of abnormal proteins in the cytosol. Although several different intracellular proteolytic systems contribute to total rates of protein degradation, lysosomes are the proteolytic system likely most affected by ageing. A decrease in macroautophagy, chaperone-mediated autophagy, and some forms of endocytosis (receptor-mediated endocytosis) occur in most tissues of old organisms[1]. Second, a microarray-based genetic screen for genes that function in the regulation of chronological lifespan in yeast revealed that a number of mutants defective for autophagy are short-lived[2]. Last, autophagy is required for the lifespan-extending effect of genetic and pharmacological manipulations in several organisms. Thus, autophagy may play an important role in delaying ageing and extending lifespan.

Autophagy and its regulation

Autophagy is a highly conserved cellular mechanism for degradation and recycle of long-lived proteins and damaged organelles. Depending on the route of cargo delivery, autophagy is classified into three types: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). Macroautophagy is the best characterized type and is the focus of this review (hereafter referred to autophagy). Our knowledge of autophagy regulation first came from the genetic study of the yeast. Up to now 35 AuTophagy-related Genes (*ATG*) have been identified in the yeast, and 16 *ATG* genes have orthologues in human[3]. These *ATG* gene products function at different stages of autophagy process and can be classified into 5 major functional groups: (i) the ULK1 kinase complex, (ii) the Atg9 cycling complex, (iii) the Vps34/class III PI3-kinase (PI3K) complexes, (iv) the lipid-binding Atg18 homolog,

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and (v) the ubiquitin-like proteins Atg12 and Atg8/LC3 and their conjugation systems[4]. The process of autophagy is composed of multiple sequential steps: induction, elongation/autophagsome formation and maturation/lysosomal degradation. Atg1/ULK1 kinase complex (Atg1, Atg13, FIP200, and Atg101) assembly at the isolated membrane called phagophore is the early event of autophagy induction[5]. Subsequently, the Beclin-1/Class III PI3K complex [including Beclin 1, PI3K Vacuolar protein sorting 34 (Vps34), p150, Atg14, UV irradiation resistance-associated tumor suppressor gene (UVRAG)] will be recruited to the isolated membrane to initialize the nucleation and elongation of autophagosome[6]. Autophagosomes are then fused with lysosomes to form autophagolysosome for degradation. Several proteins including Rab7, TECtonin β-Propeller Repeat containing 1 (TECPR1) and LAMP2 are involved in this step[7–9].

Although it normally eliminates unselectively bulky proteins or organelles, autophagy is sometimes involved in degradation of specific proteins (such as p62/SQSTM1) or organelles, such as aggrephagy (for protein aggregates), mitophagy (for mitochondria), pexophagy (for peroxisomes), reticulophagy (for ER), xenophagy (for pathogens)[10]. Autophagy can be induced in response to diverse stresses and the induction process is highly regulated. Its regulation network is complex. One of the key regulators of autophagy in mammalian cells is mTOR (mammalian target of rapamycin) kinase, which suppresses autophagy in conditions of nutrient and growth factor repletion. mTOR is activated by signal transducers including class I phoshatidylinositol-3-kinases (PI3Ks) and Akt in response to insulin, insulin-like growth factor (IGF) and other growth signals, and inhibited by AMP-activated protein kinase (AMPK) in response to reduced ATP levels[11].

Autophagy occurs constitutively at basal levels in many cell types to ensure the homoestatic turnover of long-lived proteins and organelles. Moreover, autophagy is upregulated: (i) when cells need to mobilize intracellular nutrients, as occurring during glucose and/or amino acid deprivation; (ii) when cells need to clear potentially toxic cytoplasmic materials including damaged organelles, aggregates of misfolded proteins, or invading microbes[11]; and (iii) when cells are under various stress conditions such as oxidative stress[12], ER stress[13], and proteasome inhibition[14, 15]. Technically, autophagy can be upregulated by genetic or pharmacolgical manipulations such as transgenic overexpression of sirtuin 1, knockdown of p53, administration of rapamycin, resveratrol and spermidine.

Genetic and pharmacologic manipulations that affect longevity and autophagy

Longevity and autophagy in model organisms can be affected via direct manipulation of gene expression such as knockdown or knockout of *Sirtuin 1*, *p53*, or via indirect regulation of gene expression such as administration of rapamycin, resveratrol, or spermidine. Figure 1 shows the hypothetical modes of genetic and pharmacologic manipulations in the regulation of longevity and autophagy.

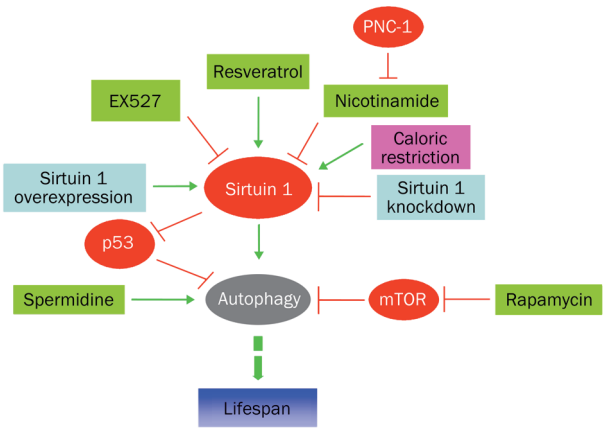

Figure 1. Hypothetical modes of genetic and pharmacologic manipulations in the regulation of longevity and autophagy. Sirtuin 1 is a nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase that can be activated by caloric restriction, by depletion of its negative regulators (such as nicotinamide that can be depleted by overexpression of pnc-1, which encodes a pyrazinamidase/nicotinamidase (*C elegan*), by pharmacological activators, in particular resveratrol. Its activity can be inhibited by pharmacological inhibitors such as EX527. Autophagy can be induced by upregulation of sirtuin 1, by downregulation of p53, or by administration of spermidine or rapamycin. These measures eventually lead to organismal longevity.

Sirtuin 1

Sirtuin 1, a phylogenetically conserved NAD^+-dependent deacetylase[16], is an important *in vivo* autophagy regulator. The transient increase in Sirtuin 1 expression is sufficient to stimulate basal rates of autophagy. Sirtuin1 can form a molecular complex with several essential components of the autophagy machinery, including autophagy related proteins (such as, Atg5, Atg7, and Atg8). It also can directly deacetylate these components *in vitro*. Sirtuin 1 knockout mice partially resemble the phenotypes of $Atg5^{-/-}$ mice, including the accumulation of damaged organelles, disruption of energy homeostasis, and early perinatal mortality. These results suggest that the Sirtuin 1 deacetylase is an important *in vivo* regulator of autophagy and provide a link between sirtuin 1 function and the overall cellular response to limited nutrients[17].

Sirtuin 1 has also been demonstrated to delay ageing and extend lifespan through inducing autophagy. Activation of the Sirtuin 1 by three different approaches (overexpression, pharmacological activation with resveratrol, and depletion of its negative regulator nicotinamide) extends lifespan through the induction of autophagy. Conversely, deactivation of Sirtuin 1 (by knockdown, knockout or pharmacological inhibition) prevents the induction of autophagy and the improvement of organismal or cellular survival by resveratrol (an indirect activator of Sirtuin 1), nutrient starvation (in human cells) or caloric restriction (in *C elegans*)[18]. Thus, Sirtuin 1 is also a major regulator of longevity in part through autophagy.

Apart from the deacetylation of autophagy-related proteins

(Atg5, Atg7, and Atg8), sirtuin 1 also deacetylates lysine residues in histone 1, histone 3 and histone 4, indicating a role for sirtuin 1 in the regulation of transcription and genomic stability via chromatin modifications[15]. In addition, sirtuin 1 controls many key pathways that are associated with its beneficial effects on metabolism and delaying ageing. They include downregulation of p53 activity, suppression of nuclear factor-κB (NF-κB)-mediated inflammatory pathways, modulation of forkhead box protein O (FOXO) transcription factors, suppression of adipogenesis pathways mediated by peroxisome proliferator-activated receptor-α (PPARα), activation of PPARα co-activator 1α (PGC1α), and promotion of insulin secretion through the suppression of mitochondrial uncoupling protein 2 in pancreatic β-cells[19]. Thus, sirtuin 1 may delay ageing and extend lifespan through other molecular mechanisms besides autophagy. Sirtuin 1 functions at a regulatory crossroad between nutrient sensing, energy metabolism and genome stability[19].

Caloric restriction may be a physiological inducer of autophagy[3] . Caloric restriction is also the only intervention that is known to retard ageing in most organisms and delay the onset of disease and functional decline in mammals[19]. Dietary restriction has been shown to extend life span in yeast, worms, flies, and mammals. Autophagy is required for dietary restriction-mediated life span extension in *C elegans* since the lifespan extending effects were compromised by knockdown of autophagy genes. Although there is a debate as to whether caloric restriction will be as effective in humans as it is in short-lived research models, data from non-human primates previously suggested that caloric restriction can improve the quality of life, reduce the risk of disease and delay mortality[19]. Interestingly, a most recent report revisits the same question and provides conflicting evidence that caloric restriction in non-human primates failed to offer any benefit in slowing ageing process[20]. Deletion of sirtuin 1 in lower organisms appears to interfere with the beneficial effects of caloric restriction in some experimental settings. However, it is noteworthy that sirtuin 1-independent lifespan extension in response to caloric restriction has also been demonstrated in yeast and worms[19]. Thus, sirtuin 1 is required for the induction of autophagy, while caloric restriction-mediated lifespan extension remains debatable especially with emerging evidence and the role of sirtuin 1 in caloric restriction mediated lifespan extension needs further clarification.

p53

P53 is a well-characterized tumor suppressor. The downregulation of p53 is associated with autophagy induction and longevity. Simultaneous promotion of lifespan extension and autophagy is also observed in *C elegans* following knockdown of the p53 orthologue, CEP-1, while the beneficial effects are abolished by depleting beclin 1/ATG6. In addition, lysine 382 of p53 is one known target of Sirtuin 1. Deacetylation of this residue by Sirtuin 1 decreases the activity and half-life of p53, accompanied by increase in cell su rvival under a variety of DNA-damaging conditions[21].

Rapamycin

Rapamycin is the best-characterized pharmacological inducer of autophagy by inhibiting TORC1. It prolongs lifespan in various organisms including mice. In worms and yeast, rapamycin extends lifespan only when autophagy is induced. Rapamycin cannot extend the chronological lifespan of yeast mutants that lack the essential autophagy genes *ATG1* or *ATG7*[22]. In *C elegans*, the beneficial effects of rapamycin on longevity are lost when the essential autophagy genes *bec-1* (the worm ortholog of mammalian gene *Atg6/beclin 1*) or *vps34* are knocked down[23]. These results indicate that rapamycin induce lifespan extension by inducing autophagy in *worms* and yeast. In mice, it remains to be determined whether rapamycin prolongs the lifespan of mice by inducing autophagy, although rapamycin fed late in life extends lifespan in genetically heterogeneous (out-bred) mice. Rapamycin may extend lifespan by postponing death from cancer, by retarding mechanisms of ageing, or both[24]. Rapamycin-induced autophagy is independent of sirtuin 1 in human cells and in *C elegans*, suggesting that rapamycin and sirtuin 1 promote autophagy through distinct, non-overlapping mechanisms[25].

Resveratrol

Resveratrol is a polyphenol mainly found in red grape skin. It has been shown to extend the lifespan in diverse organisms such as yeast, worms and flies[21, 26]. An additional study showed that resveratrol extends lifespan in a short-lived species of fish[19]. It also improves health and survival of mice on a high-calorie diet[27]. However, it is noteworthy that sirtuin 1 overexpression or resveratrol fails to extend the lifespan of mice on a normal diet. Resveratrol significantly increased the level of sirtuin 1 in diverse cells including human cells, but failed to extend lifespan under the condition of the lack of sirtuin 1. These results indicate that the lifespan-extending effect of resveratrol is dependent on sirtuin 1[19, 21].

As discussed above, sirtuin 1 is an important regulator of autophagy and longevity. Resveratrol also induces autophagy in human cancer cells and *C elegans*, and the effect was fully prevented with genetically impaired *sirtuin 1* or treatment of pharmacological inhibitor (EX527)[28]. Resveratrol only prolonged the lifespan of autophagy-proficient *C elegans*, whereas these beneficial effects on longevity were abolished by the knockdown of the essential autophagic modulator beclin 1. Although it may extend lifespan through pathways such as suppressing rDNA recombination[21], resveratrol has been shown to promote longevity (or at least partly) through sirtuin-1-dependent induction of autophagy[28].

It was shown that resveratrol has many effects that are consistent with sirtuin1 activation. For example, it improves insulin sensitivity, inhibits tumour growth, suppresses inflammation, promotes cardiovascular health and protects against neurodegenerative diseases[19].

Spermidine

The decreases in the levels of the main polyamines (putrescine and spermidine) were observed in different mammalian

organs with ageing[29]. The spermidine has also been shown to delay ageing and promote longevity in yeast, flies and worms. A diet enriched in physiologically relevant polyamines (*eg* putrescine, spermidine and spermine) also increases lifespan and health span in mice[30].

Spermidine treatment also induces autophagy in model systems including yeasts, flies, nematodes and mammalian cells. Polyamine depletion decreases yeast lifespan and increases necrosis. Spermidine-mediated lifespan extension was abolished in yeast, flies and worms if autophagy is blocked by knockout or knockdown of the essential autophagy genes, *ATG7, BECN1*. These results indicate that spermidine promotes ageing-delaying or lifespan extension through autophagy-dependent manner[31].

Deletion of sirtuin 1 or any other sirtuin did not abrogate the ability of spermidine to extend chronological lifespan, indicating that spermidine promotes autophagy and then induces lifespan extension by other pathways rather than sirtuin 1. Spermidine inhibits histone acetylase, while resveratrol activates the histone deacetylase sirtuin 1 to confer cytoprotein/longevity. These results indicate the essential role of protein hypoacetylation in autophagy control and in the regulation of longevity.

Autophagy and ageing-related diseases

Autophagy plays vital roles in multiple biological functions from development to cell survival. Dysfunction of autophagy has been linked to a variety of ageing-related diseases including cancer and neurodegenerative diseases[32].

Autophagy and cancer

Emerging evidence reveals the connection between autophagy and cancer. One of the most important lines of evidence came from the study of the autophagy gene *BECN1*. Monallelic deletion of *BECN1* was associated with high frequency of tumors in multiple tissues including breast, ovarian and prostate[33]. Heterozygous deletion of *BECN1* mice showed increased susceptibility to multiple tumors[34, 35]. However, there is also evidence that autophagy is a mechanism by which solid tumor cells survive from hypoxic and metabolic stresses[36, 37]. Despite recent efforts, it is still inconclusive about what exact role autophagy plays in the different stages of carcinogenesis.

Early studies suggested that autophagy suppresses the initiation of tumorigenesis. In addition to *BECN1* gene that is monoallelic deleted in multiple cancers, genetic deletions or mutations in other autophagy associated genes including *UVRAG, ATG2B*, *ATG5*, *ATG9B*, and *ATG12*, were also observed in multiple types of cancers[37, 38]. These reports highlight the genetic links between autophagy and tumorigenesis. It is not clear how autophagy defects may contribute to the initiation of tumor; at least two hypothetic mechanisms may be involved. First, autophagy maintains cellular homeostasis by constantly digesting misfolded proteins and damaged organelles (*eg* mitochondria), while disturbing the normal function of autophagy leads to accumulation of harmful metabolic end products, oxidized molecular and damaged organelles. As consequence of disrupted autophagy, the cells suffer damages in chromosomal DNA, causing genomic instability. Second, inhibition of autophagy may deregulate cell proliferation process or stimulate necrotic cell death, which cause release of cellular contents to the surrounding environments and initiate inflammation responses that promote tumor developments[39].

In addition, autophagy can also serve as a survival mechanism for tumors. Tumors are basically under increased hypoxia stress and nutrition deprivation due to rapid proliferation rate and limited blood supply[40]. Under extremely harsh metabolic stress, tumors rely on autophagy to provide nutrient and keep survival[41] . For certain cancer types with apoptosis defects, autophagy is an important survival mechanism to keep cell viability through long-term metabolic stresses such as glucose deprivation and hypoxia[36, 42].

Targeting autophagy as therapeutical strategy against cancer attracts wide interests. It is not only because mounting genetic evidence links autophagy to cancer, but also multiple anticancer drugs indeed activate autophagy response (For details please refer to previous review[43]). Autophagy inhibitors such as 3-MA and hydroxychloroquine sensitize tumors to the anti-cancer drugs, while autophagy inducers like rapamycin inhibits tumor proliferation[44] and sensitizes tumors to the radiation[45]. Current knowledge supports the view that modulation of autophagy can be a therapeutic strategy against cancers. Future work will be needed to determine the time points and direction (induce or inhibit) of autophagy modulation for different types of tumors and at different stages.

Autophagy and neurodegenerative diseases

Neurodegenerative diseases are pathological conditions in nervous system characterized by progressive neuron loss, normally accompanied by accumulation of abnormal protein aggregates in the affected regions. The initial observation linking autophagy to neurodegenerative diseases is the presence of abnormal autophagosomes in the affected neurons of neurodegenerative diseases[46]. However, it is unclear whether the autophagosome formation is a consequence of impaired autophagic clearance or enhanced autophagy induction. Furthermore, it remains to be determined under those conditions whether neuronal autophagy represents protective or deleterious mechanisms. Two seminal studies using genetic mouse models lacking essential autophagy genes in the brain unequivocally demonstrate neuroprotective function of basal autophagy[47, 48].

Protein aggregation, a common feature of several major neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's disease, is the consequence of accelerated protein deposition or/and reduced turnover. Numerous disease-related protein species tend to form toxic oligomers that evoke a wide range of cell stresses including calcium flux, ER stress and oxidative stress that eventually lead to neuronal loss. Our study using the transgenic mouse model with essential autophagy gene (*Atg7*) deletion in TH positive neurons suggests that impairment of autophagy may be associated

with accumulation of endogenous α-syn and LRRK2 proteins in the dopaminergic neurons, implying that dysfunction of autophagy may be one of the pathogenic mechanisms of PD[49]. The emerging evidence that autophagy is neuroprotective through the clearance of unwanted protein complexes (including disease protein oligomers) makes autophagy a promising drug target, and indeed inspired a new round of drug discovery for the autophagy enhancers as therapeutic agents against neurodegenerative diseases such as Parkinson's disease and Huntington's disease. A number of chemical autophagy inducers have been identified to promote the clearance of pathogenic protein aggregates relevant to neurodegenerative diseases (summarized in Table 1).

Neurons are highly specialized cell type which relies on vigorous autophagy to remove misfolded proteins and damaged organelles for keeping viability. The regulation of autophagy in the neurons is different from other cell types, given the observations that: i) lipidation form of LC3 and autophagosomes are scarce in-neurons at basal level[65]; ii) nutrition starvation, the most robust autophagy inducer for most cell types, poorly induce autophagy in-brain[66]. Understanding the regulatory mechanism of neuronal autophagy will enable the design and discovery of the neuron-specific autophagy enhancers which regulate the autophagic activity within safe range. However, autophagy is a complex process including initiation, elongation and maturation steps, and thus simply evoking the formation of autophagosome may not be sufficient for treating the diseases with defects in cargo turnover such as AD and frontotemporal dementia[67, 68]. It is thus desirable to restore the lysosome function while inducing autophagy biosynthesis. Thus combination of autophagy inducers and lysosome biogenesis enhancers is a better strategy for fighting the neurodegerative diseases.

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Table 1. Autophagy modulator in neurodegenerative disease models.

Autophagy inducer	Mechanism of action	Neurodegenerative disease models
Rapamycin	mTOR inhibition	Reduction of polyglutamine and polyalanine aggregation[45]; Ameliorates polyglutamine aggregates and toxicity in animal models[46]; Alpha-synuclein degradation[47]; Ameliorates cognitive deficits and reduces amyloid-beta AD mouse model of Alzheimer's disease[48].
Small molecular enhancers (SMERs)	mTOR-independent	Mutant Huntingtin (Htt) and mutant A53T α-synuclein clearance[49].
Lithium	Inositol Monophosphatase Inhibition	Mutant Htt aggregation/toxicity amelioration[49].
N10-substituted phenoxazine	mTOR-independent	Decreased the accumulation of diffuse and aggregated Htt[50]; Mutant Htt and α-synuclein reduction[51, 52]; Ameliorates dopaminergic and tau pathology[53].
Trehalose	mTOR-independent	Enhanced cellular degradation of prions[54].
Latrepirdine	mTOR-dependent	Improved learning behavior and reduction of Aβ42 and α-synuclein[55, 56].
Corynoxine B	mTOR-independent, Beclin 1-indepent	WT and mutant α-synuclein reduction[57, 58].
17-AAG	Unknown	Wild type and mutant α-synuclein reduction[59].

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