Autophagy and ageing: implications for age-related neurodegenerative diseases

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Abstract

Autophagy is a process of lysosome-dependent intracellular degradation that participates in the liberation of resources including amino acids and energy to maintain homeostasis. Autophagy is particularly important in stress conditions such as nutrient starvation and any perturbation in the ability of the cell to activate or regulate autophagy can lead to cellular dysfunction and disease. An area of intense research interest is the role and indeed the fate of autophagy during cellular and organismal ageing. Age-related disorders are associated with increased cellular stress and assault including DNA damage, reduced energy availability, protein aggregation and accumulation of damaged organelles. A reduction in autophagy activity has been observed in a number of ageing models and its up-regulation via pharmacological and genetic methods can alleviate age-related pathologies. In particular, autophagy induction can enhance clearance of toxic intracellular waste associated with neurodegenerative diseases and has been comprehensively demonstrated to improve lifespan in yeast, worms, flies, rodents and primates. The situation, however, has been complicated by the identification that autophagy up-regulation can also occur during ageing. Indeed, in certain situations, reduced autophagosome induction may actually provide benefits to ageing cells. Future studies will undoubtedly improve our understanding of exactly how the multiple signals that are integrated to control appropriate autophagy activity change during ageing, what affect this has on autophagy and to what extent autophagy contributes to age-associated pathologies. Identification of mechanisms that influence a healthy lifespan is of economic, medical and social importance in our ‘ageing’ world.

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Introduction

Three types of autophagy

Autophagy is characterized by the lysosomal degradation of substrates, however, the type of substrate as well as the mode of substrate recognition, transport and delivery differentiates the three known types of autophagy. First, macroautophagy is the term given to the isolation of cytoplasmic substrates into a double-membrane-bound organelle called an autophagosome. Autophagosomes are transported along the microtubule cytoskeleton network and their contents are degraded following fusion with the lysosome. A number of protein complexes have been demonstrated to be essential for macroautophagy induction. The ULK1 (uncoordinated-51-like kinase 1)-containing complex, the class III PI3K (phosphoinositide 3-kinase)–Beclin1 complex and the Atg5–Atg12–Atg16L1 (autophagy related 16-like 1) complex are indispensable for macroautophagy and participate in the initiation and growth of autophagosomes. Formation of an autophagosome also requires lipidation of LC3 (light-chain 3 or microtubule-associated protein 1 light chain 3; Atg8 in yeast) which is frequently used as a marker of macroautophagy. In addition, transport and fusion of autophagosomes with lysosomes is facilitated by a number of known proteins including Rab7 and SNAREs (soluble N-ethylmaleimide-sensitive fusion protein-attachment protein receptors) (Figure 1) [1]. Secondly, CMA (chaperone-mediated autophagy) utilizes the chaperone protein Hsc70 (heat-shock cognate 70 stress protein) to recognize specific soluble protein substrates. These substrates are delivered to the lysosomal membrane where they are unfolded and translocated into the lysosomal lumen via a Lamp2a-dependent mechanism (Figure 1) [2]. Thirdly, microautophagy is a process whereby cytoplasmic substrates are directly engulfed by lysosomes via invagination of the membrane (Figure 1) [3]. Little is known about how substrates are targeted for microautophagy and, furthermore, its activity during ageing has not been investigated to date. We will therefore limit the discussion in the present chapter to macroautophagy and CMA.

Upstream regulation of autophagy

The molecular mechanisms that regulate CMA are not well understood although increased activity has been observed in response to nutrient starvation, oxidative stress and hypoxia [2]. The mechanisms of macroautophagy regulation on the other hand have been studied in much greater detail. Nutrient starvation is a potent activator of autophagy and this occurs via the inhibition of the serine/threonine kinase mTOR [mammalian (also known as mechanistic target of rapamycin) which occurs in complex with regulatory proteins [mTORC1 (mTOR complex 1)]. Integration of a number of mitogenic and growth-promoting signalling pathways converge on this core regulator, the best studied is the insulin–PI3K–Akt signalling axis [1].

Macroautophagy can also be activated in response to low-energy availability and is mediated by AMPK (AMP-activated protein kinase) and members of the SirTuin family of deacetylases. Specifically, AMPK can induce macroautophagy via the inactivation of mTORC1 as well
The most widely studied member of the Sirtuin family SIRT1 promotes macroautophagy by direct deacetylation of the Atg (autophagy-related) proteins Atg5, Atg12 and LC3 [5]. Both AMPK and SIRT1 can also activate the transcription factor FoxO3a which promotes the expression of a number of autophagy-related genes including LC3, Atg12, Bnip3 and Rab7 [6,7]. In addition, the effect of SIRT1 on macroautophagy can be mediated by the transcription factor p53 which can both induce (via transcriptional control of antioxidant Sestrin proteins that activate AMPK) and inhibit (when present in the cytoplasm) the process [6,8]. Other, mTOR-independent mechanisms regulating macroautophagy have also been identified, such as those involving free inositol levels in the cell [1,9].

as by direct phosphorylation of the autophagy-activating serine/threonine kinase ULK1 [4]. The most widely studied member of the Sirtuin family SIRT1 promotes macroautophagy by direct deacetylation of the Atg (autophagy-related) proteins Atg5, Atg12 and LC3 [5]. Both AMPK and SIRT1 can also activate the transcription factor FoxO3a which promotes the expression of a number of autophagy-related genes including LC3, Atg12, Bnip3 and Rab7 [6,7]. In addition, the effect of SIRT1 on macroautophagy can be mediated by the transcription factor p53 which can both induce (via transcriptional control of antioxidant Sestrin proteins that activate AMPK) and inhibit (when present in the cytoplasm) the process [6,8]. Other, mTOR-independent mechanisms regulating macroautophagy have also been identified, such as those involving free inositol levels in the cell [1,9].

Figure 1. Overview of autophagy

Autophagy is a generic name for three major pathways of lysosome-dependent degradation of cytoplasmic components. Macroautophagy involves the sequestration of cytoplasmic contents including proteins and entire organelles into a double-membrane-bound autophagosome. Macroautophagy induction is regulated by a number of multiprotein complexes required for proper initiation, elongation and maturation of autophagosomes. These are transported along the microtubule network and fuse with lysosomes in a Rab7- and SNARE-dependent manner delivering their contents for hydrolytic degradation. CMA involves the recognition of protein substrates via a consensus peptide motif, KFERQ or similar, by the chaperone protein Hsc70. The protein substrates are delivered to the lysosome where they are unfolded and translocated across the lysosomal membrane into the acidic lumen via a Lamp2a-dependent mechanism. Microautophagy allows delivery of cytoplasmic contents to the lysosome lumen via direct invagination of the lysosome membrane.
Autophagy and ageing

Ageing is characterized by the accumulation of detrimental molecular, cellular and functional changes to an organism. It is associated with a gradual deterioration in cellular fitness and viability and an increase in cellular transformation. Age-related dysregulation of autophagy has been observed and, equally, dysregulated autophagy can contribute both directly and indirectly to cellular damage, such as an accumulation of damaged or toxic aggregates and organelles, thus compounding the problems associated with age. In the present chapter we discuss the current understanding of how ageing may cause a dysfunction in autophagy potential, including changes in transcription, translation and function of autophagy-related genes. We will also describe how modulation of autophagy can affect the ageing phenotype with a special focus on the role of autophagy in age-related neurodegenerative diseases.

Observed changes in autophagy during ageing

Altered expression and function of autophagy-related genes

Although ageing is often associated with a decrease in autophagy potential, current evidence does not paint a consistent picture as to the consequences of ageing on the expression of autophagy-related proteins (Figure 2). For example, both elevated [10] and decreased [11] expression of Beclin1 has been observed in aged tissues. Similar inconsistencies have also been observed in the expression of LC3, Atg5 and Atg7 [10–12]. In addition to altered expression of proteins, functional defects have also been observed during ageing. For example, age-related accumulation of lipofuscin can also significantly impair lysosomal function thereby reducing macroautophagy- and CMA-dependent substrate clearance. CMA is also impaired in aged rats, not as a result of decreased Lamp2a expression, but rather due to changes in the lysosomal membrane which causes a reduction in Lamp2a localization [13,14]. Appropriate levels of functional autophagy indeed contribute to maintaining health during ageing as demonstrated when Lamp2a levels are maintained into old age which preserves CMA levels in mice and ameliorates ageing-related phenotypes [13]. An area that has yet to be fully explored in ageing cells is the cross-talk and potential compensation mechanisms between the different modes of autophagy and the UPS (ubiquitin–proteasome system). Such cross-talk is important for the regulation of cellular homoeostasis. For example, macroautophagy is up-regulated in cells with defective CMA [15] and autophagy is up-regulated when the UPS system is impaired [16]. Specific cellular assaults and (dys)function during ageing are likely to contribute to the observed alterations in autophagy-related protein expression and function. A better understanding of the underlying mechanisms of ageing will undoubtedly help to elucidate the cause and effect of these changes in autophagy potential.

Reduced responsiveness to external and internal stimuli

The ability to sense and respond appropriately to external and internal stimuli is essential to maintain cellular homoeostasis. With increasing age, however, the responsiveness and activity of AMPK and SIRT1 are decreased thereby directly and indirectly contributing to the
dysregulation of autophagy (Figure 2). A number of studies have demonstrated that AMPK is not activated in old tissues under conditions where young tissues show a robust response [7]. The mechanisms of AMPK activity reduction are not well understood, but altered expression and activity of positive and negative regulators are likely to be important factors. SIRT1 activity may also decrease with age via decrease in expression [17] and decreased intracellular NAD$^+$ levels [18], leading to decreased cellular antioxidant capacity. Indeed, oxidative stress is particularly important during ageing and, in addition to SIRT1, other oxidative stress and autophagy regulators such as the polyamine spermidine are also reduced during ageing [19]. Reduction in FoxO3a [20] and p53 [7,21] activity have also been observed in ageing rodents which may also contribute to dysregulated autophagy (Figure 2).

**Increased cellular damage and inflammatory responses**

Low-grade chronic inflammation is a hallmark of ageing; however, there is a complex cause-and-effect relationship between inflammation, cellular damage and ageing, and the participation of
autophagy in this process is under debate. The pro-inflammatory transcription factor NF-κB (nuclear factor-κB) is activated in response to cellular stress and DNA damage and its activity is increased in normal and accelerated ageing models [22]. Genetic or pharmacological inhibition of NF-κB can reduce ageing-related cell damage. The activity of the autophagy-inhibiting NF-κB is inversely correlated with the activity of the autophagy-activating AMPK and SIRT1 which, as discussed above, are reduced during ageing. Cross-talk between these signalling pathways and autophagy has yet to be fully investigated in ageing models. However, the careful balance between and maintenance of cellular stress responses is likely to be an important contributor to cellular ageing mechanisms. Indeed, increased age-related ROS (reactive oxygen species) for example, enhances cellular oxidative stress, inflammation, DNA damage and dysregulated mitochondria (Figure 3) [18,23,24]. Importantly, dysregulation of mitochondria causes a chronic reduction in cellular energy production, increased autophagy and reduced antioxidant capacity. This directly contributes to the inflammatory phenotype via further production of ROS [24] and ageing phenotypes. With regard to autophagy, on one hand, an increase in damaged mitochondria and ROS can promote autophagy up-regulation in an attempt to clear the damaged organelles. However, the extent to which autophagy can be activated is dependent on the expression and availability of its regulators, which as we have discussed can be altered during ageing. Furthermore, ROS production can also disrupt autophagy machinery, leading to a reduced capacity for autophagy clearance. Further work is required to elucidate the relationships between ROS, mitochondrial dysfunction and autophagy. It is clear that a wide range of complex interdependent relationships participate to tightly control cellular stress responses and maintain energy (Figures 2 and 3).

**Figure 3. The general effect of dysregulated autophagy on cellular fate**

Dysregulation of autophagy can have an impact on a variety of processes that ultimately affect cell health and survival. It is important to note that there are complex cause-and-effect relationships between many ageing-related cellular changes. For example, an accumulation of damaged mitochondria can contribute to increased intracellular ROS levels that can directly affect inflammatory responses as well as mitochondrial health and clearance. Such relationships can further compound age-related pathologies and have a direct impact on the regulation of autophagy.
Investigating the underlying mechanisms regulating inflammation, ROS, mitochondrial dysfunction and autophagy are important considerations for the future of ageing research.

**Up-regulation of macroautophagy during ageing?**

Reduced autophagy-related protein expression, function and responsiveness have all been suggested to contribute to cellular ageing. The situation, however, is not straightforward and in aged tissues an increase in macroautophagy has also been observed. Specifically, an increase in the expression of the co-chaperone protein BAG3, at the expense of BAG1, has been observed during ageing representing a shift from UPS- to macroautophagy-dependent degradation [25]. BAG3 can influence autophagy activity by interacting with the microtubule motor dynein and an adaptor protein called p62. It has been suggested that proteins destined for degradation are incorporated via BAG3 into p62-positive protein aggregates and targeted to autophagosomes [25,26]. Furthermore, BAG3 with a binding partner HspB8 can activate eIF2α and directly influence autophagy induction [27]. In addition, activation of autophagy has been observed in the mouse model of premature ageing, Hutchinson–Gilford progeria, and is associated with increased AMPK activity and decreased mTOR activity [28].

Additional work is required to further understand how autophagy regulation is altered during ageing. Specific environmental and genetic factors are likely to contribute to the precise modifications of autophagy-related protein expression and function during ageing. There is increasing evidence, however, that modulation of autophagy-related pathways can affect ageing and that carefully controlled autophagy could alleviate ageing-related pathologies and promote extension of a healthy lifespan.

**The contribution of autophagy to ageing**

**Participation of autophagy in cellular senescence**

Cellular senescence is often studied as a cell culture model of ageing although at an organismal level senescence can not only promote, but also prevent ageing by acting as a tumour suppression mechanism. Senescence refers to cells that have irreversibly exited the cell cycle (i.e. post-mitotic), but remain metabolically active. It is considered to be a cell survival mechanism in the face of irreparable cellular damage that would otherwise lead to cellular transformation. Senescence can occur via replicative (telomere shortening) or non-replicative (DNA damage response or oncogene activation) mechanisms. Senescent cells are characterized by an increase in size and a SASP (senescence-associated secretory phenotype) mediated by secretion of cytokines and growth factors [29].

It is unclear at present what the true contribution of senescence to ageing is and indeed the extent to which autophagy contributes to senescence (Figure 3). For example, activation of autophagy has been shown to contribute to senescence in some studies of OIS (oncogene-induced senescence); mTORC1 activity is reduced, autophagy genes are up-regulated and genetic inhibition of autophagy delays the senescent phenotype [30]. However, in other studies, the mTORC1 inhibitor rapamycin has been shown to slow onset of senescence [31], possibly implicating reduced autophagy in senescence. Cellular senescence is an expanding field that in recent years has shifted focus from a molecular damage-induced (e.g. DNA damage and ROS production) senescence model to an mTORC1-centric model whereby mTORC1 stimulates ageing via hyper-activation of a cellular growth-promoting programme. The two models,
however, are not necessarily mutually exclusive and future work in this area will certainly contribute important understanding to the role of mTOR and autophagy in senescence and the contribution of senescence to ageing.

**Induction of autophagy contributes to increased lifespan**

Studies designed to investigate factors that contribute to ageing have consistently identified proteins and pathways that modulate autophagy. One of the first mechanisms identified to increase lifespan was dietary restriction. The lifespan-promoting effect of this intervention appears to be evolutionarily conserved and has been observed in yeast, worms (*Caenorhabditis elegans*), flies (*Drosophila melanogaster*), rodents and primates. This increased longevity is mediated, at least in part, by the activation of autophagy, as lifespan extension is prevented by deletion or mutation of autophagy-related genes [32]. For example, mutation in the *C. elegans* Beclin1 homologue *bec-1* prevents the life-extending benefits of *daf-2* [insulin/IGF-1 (insulin-like growth factor-1) receptor] mutations [33]. Activators of autophagy including rapamycin, resveratrol and spermidine can also promote increased lifespan [19]. Cellular mTORC1 activity is important for longevity; it has been observed to persist during normal ageing and genetic or pharmacological down-regulation of the IGF-1–Akt–mTORC1 signalling axis can promote increased lifespan [34] and deletion of the downstream S6 protein kinase can extend lifespan of mice possibly via AMPK activation [35]. Modulation of upstream regulators of mTORC1 and autophagy provide further weight to the important contribution of autophagy to ageing. For example, activation of the autophagy-promoting proteins AMPK, SIRT1 and FoxO3a can also promote lifespan extension and inhibition of their activity can enhance ageing phenotypes [36], whereas loss of the pro-inflammatory p53 homologue in *C. elegans* can increase lifespan [37]. Specifically, SIRT1 may play a protective role to limit the detrimental effects of stressors such as ROS, as well as contributing to autophagy induction via autophagosome formation [6]. Indeed, lifespan extension mediated by dietary restriction is associated with increased SIRT1 expression and SIRT1 activators such as hypoxia can activate autophagy. The central importance of autophagy to promoting lifespan is further emphasized when one considers that different mechanisms of longevity, i.e. calorie restriction compared with rapamycin-induced are all ultimately dependent on autophagy-related genes.

**Autophagy in age-related neurodegeneration**

Autophagy is particularly important in post-mitotic or terminally differentiated cells such as neurons as they are unable to use ‘mitotic dilution’ as an option to remove toxic aggregates. Instead they rely on functional quality control mechanisms including autophagy and the UPS to remove potentially damaging proteins, complexes or entire organelles and are therefore extremely vulnerable when such quality control mechanisms go wrong. Parkinson’s disease and Huntington’s disease are associated with cytoplasmic aggregation of α-synuclein and mutant huntingtin respectively, and impaired function of autophagy pathways (Figure 4). For example, wild-type α-synuclein is ordinarily degraded by CMA and although Parkinson’s disease-associated mutant α-synuclein can still be recruited to lysosomes, it is not translocated to the lysosome lumen. Furthermore, its association with CMA receptors subsequently impairs the ability of other substrates to bind and be degraded [38]. Genetic or
Figure 4. Autophagy and neurodegeneration
Dysregulation of autophagy in neurons has been implicated in neurodegenerative diseases including HD (Huntington’s disease), AD (Alzheimer’s disease) and PD (Parkinson’s disease). The incidence of such diseases increases with age and many of the age-associated perturbations in autophagy regulation described in the present chapter are also associated with neurodegeneration. A particularly important relationship between ageing, neurodegeneration and autophagy is protein aggregation and its downstream consequences. For example, mutant tau proteins are observed in AD to cause neurofibrillary tangles thus perturbing membrane trafficking and it can block CMA machinery and thereby reduce overall CMA. In HD, expansion of the N-terminus of Htt (huntingtin) by multiple glutamine residues results in its accumulation and dysregulation of autophagy via multiple mechanisms. For example, autophagy perturbation can be caused by sequestration of Beclin1 into Htt aggregates; mutant Htt has also been shown to prevent recruitment of substrates to autophagic vesicles. In PD, mutant α-synuclein is recruited to CMA receptors, however, it is not translocated into the lysosome lumen. As a result, CMA receptors are unable to bind any further substrates and its activity is impaired. Dysfunctional mitochondria are associated with PD and AD. Mutations in the autophagy-associated mitochondrial proteins PINK and parkin prevent the clearance of damaged mitochondria in PD. Reduced expression (e.g. Beclin1 in AD and HD) and localization (Lamp2a) of proteins can perturb autophagy in ageing and neurodegenerative diseases. In AD cases caused by presenilin mutations, an increase in the number of cytoplasmic autophagosomes has been observed, possibly as the result of reduced lysosome acidification and therefore function. The accumulation of autophagosomes further contributes to AD pathology as a source of amyloid-β, which accumulates as a result of cleavage of the APP (amyloid precursor protein) and is secreted into the extracellular space where it forms amyloid plaques, a hallmark of AD.
pharmacological induction of autophagy can help to alleviate symptoms of neurodegenerative disorders by promoting degradation of mutant proteins in various animal models and increase lifespan [39].

Dysfunctional autophagy in neurodegenerative diseases can result from reduced expression and function of autophagy proteins (Figure 4). For example, a reduction in Beclin1 expression has been noted in patients suffering from Alzheimer’s disease and Huntington’s disease. Beclin1 can also be sequestered into mutant huntingtin aggregates and is thus unable to induce autophagy. Increasing the expression of Beclin1 allows restoration of functional autophagy and clearance of toxic proteins thus improving cellular pathology [38]. Mutations causing neurodegenerative diseases can also directly contribute to the impairment of autophagy. For example one of the consequences of Huntington’s disease-causing polyglutamine expansion in huntingtin protein is defective autophagic substrate recruitment to autophagosomes. Indeed, a knock-in of mutant huntingtin lacking this polyglutamine tail, improves disease symptoms, promotes autophagosome formation and extends lifespan [40]. Furthermore, mutations of the ubiquitin ligase parkin and the serine/threonine kinase PINK1 [PTEN (phosphatase and tensin homologue deleted on chromosome 10)-induced putative kinase 1] are observed in sufferers of Parkinson’s disease. These proteins are both autophagy-related proteins that participate in the specific targeting of damaged mitochondria for autophagic degradation and their mutation is associated with reduced autophagic clearance of mitochondria, increased mitochondrial dysfunction and oxidative stress thereby directly contributing to disease pathology [32,39,41].

In addition to protein aggregation and mitochondrial dysfunction, dysregulated autophagy in neurodegenerative diseases has also been observed as a result of reduced lysosome dysfunction. In Alzheimer’s disease, a reduction in lysosomal lumen acidification has been associated with an accumulation of autophagosomes [42]. This is particularly important as autophagosomes have been implicated in the accumulation and subsequent extracellular deposition of amyloid-β which directly contributes to the disease pathology. Alzheimer’s disease is also associated with dysfunctional hyper-phosphorylated tau proteins which ordinarily regulate microtubule dynamics, but in disease states contribute to neurofibrillar tangles and impaired membrane trafficking. Indeed neurons are particularly sensitive to impaired membrane trafficking events due to their elongated shape and defects in the microtubule network can have negative effects on the rate of autophagosome clearance. In such situations, improvements in lysosomal clearance or even inhibition of autophagosome synthesis have been suggested to be more beneficial than up-regulation of autophagy [43].

**Conclusion**

Dysregulated autophagy has been observed in ageing and age-related diseases and is an important mediator of pathology. Since the first demonstrations of a potential protective role for autophagy during ageing, many contributory and antagonistic regulatory mechanisms have been discovered. The potential to exploit autophagy as a therapeutic target for age-related pathologies including neurodegeneration is an attractive prospect. The challenge still remains, however, to identify medical interventions that promote appropriate levels of autophagy to maintain cellular homoeostasis.
Summary

- Dysregulation of autophagy has been observed in ageing tissues and is associated with altered expression and function of regulatory proteins and signalling pathways.
- Although many studies suggest autophagy is reduced during ageing, it must be noted that up-regulation of autophagy activity has also been demonstrated during ageing. It is likely that many factors contribute to the tight spatial and temporal activity of autophagy.
- Pharmacological and genetic inhibition of the insulin–Akt–mTORC1 signalling pathway is associated with increased lifespan via an evolutionarily conserved mechanism from yeast, to worms, flies, rodents and primates. These effects are abrogated in the absence of autophagy highlighting the central importance of this catabolic process in the promotion of lifespan.
- Neurodegenerative disorders such as Alzheimer’s, Huntington’s and Parkinson’s diseases are often characterized by accumulation of protein aggregates. Dysregulated autophagy has been implicated in these pathologies and activation (less frequently suppression) of autophagy has been proposed as a promising therapeutic strategy.

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References


