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Autophagy in Aging and Alzheimer's Disease: Pathologic or Protective?

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Abstract

Some hypothesize that aging in humans is a cumulative process of macromolecular and mitochondrial damage starting years, even decades before any symptoms arise. Aging may begin when the rate of damage exceeds the rate of continual repair and turnover. Quality control for damaged mitochondria entails cellular digestion by mitophagy, a specialized kind of autophagy. Insufficient protective autophagy could cause damaged cellular components to accumulate over many years until they affect normal function in the cell. Alternatively, aging could be the result of overactive, pathologic autophagy. Current knowledge supports both hypotheses with conflicting data, depending on which stage of autophagy is examined. To distinguish these opposite hypotheses, two criteria need to be observed. First, is there a buildup of undigested waste that can be removed by stimulation of autophagy? Or second, if autophagy is overactive, does inhibition of autophagy rescue cell, organ and organism demise. Both of these are best determined by rate measures rather than measures at a single time point. Here, we review the generalized process of autophagy, with a focus on the limited information available for neuron mitophagy, aging and Alzheimer's disease. In two mouse models, treatment with rapamycin abolishes the AD pathology and reverses memory deficits. As a working model, we hypothesize that insufficient protective autophagy accelerates both aging and Alzheimer's disease pathology, possibly caused by defects in autophagosome fusion with lysosomes.

Keywords

autophagy; mitochondrial turnover; beclin; mTOR; autophagosome; lysosome

Autophagy is a catabolic process utilized by all cells to clear damaged proteins and organelles and aid in adjusting to changes in the environment. In contrast to small molecules, degraded by microautophagy and chaperone mediated autophagy, macroautophagy digests entire organelles[1]. The turnover of organelles by macroautophagy is vital to organ development and maximal cellular function. Mitophagy is the subform of macroautophagy that mediates removal of damaged mitochondria. Damage to mitochondria manifests in both general aging, [2] as well as in Alzheimer's disease[3], two populations of increasing demographic importance. The oxidation and reduction reactions abundant in mitochondria cause oxidative damage requiring constant turnover to prevent accumulation of damaged organelles.

Autophagy is phylogenetically conserved from yeast to mammals. Most of the elucidation of the 29 Atg genes and proteins in autophagy has first been identified in yeast, with extension to 16 mammalian orthologues largely by genetic homology (Table 1). A number of mammalian orthologues have multiple isoforms [4, 5]. For example, the Atg8 mammalian orthologue has been found to have at least 7 organ-specific isoforms [6]. Further work is needed to identify others and their region and stimulus-specific functions. Here, we focus on environmental stimuli of mitophagy especially in the context of aging and Alzheimer's disease.

Macroautophagy can be broken down into 2 forms, quality control (QC) autophagy and starvation induced autophagy. QC autophagy is present in normal cell function to degrade proteins and organelles that become mutated, misfolded, or oxidized and recycle the remaining component lipids, nucleic acids, amino acids and micronutrients back to the cytosol as nutrients. Damaged cytosolic components are selected for degradation by QC autophagy through ubiquitin tags. Histone deacetylase 6 (HDAC6) binds ubiquitinated proteins and organelles, selecting them for degradation [7]. Starvation-induced autophagy does not require ubiquitination of substrates for degradation. At least, an HDAC6 knock-out does not inhibit autophagosome formation under nutrient deprivation. Starvation-Induced autophagy is regulated through mammalian target of rapamycin (mTOR) inhibition to compensate for limited nutrients in the environment [8]. Macroautophagy is also regulated through mTOR by nutrient supply and environmental toxins that cause intracellular damage; mTOR is itself the target of multiple signaling pathways. To ensure that autophagy does not become overactive and cytotoxic, certain amino acids released from protein degradation act as negative feedback regulators [9].

As cells age, autophagy decreases [10], causing the buildup of defective proteins and organelles and eventually cell death. Malfunctions in quality control degradation are especially harmful in cells that aren't readily replenished by mitotic cycles, such as neurons and muscle. The malfunction of autophagy is a key element in the pathology of several neurodegenerative diseases including Alzheimer's and possibly aging. Either excessive or inadequate autophagy could be triggers for both aging and Alzheimer's disease. After reviewing the regulation and normal functions of autophagy and mitophagy, we will delineate approaches to discern whether too much or too little autophagy prevails in aging and Alzheimer disease.

1. Normal Functions of Autophagy

Although QC autophagy is present in all cell types, it is more important in cells that do not divide after differentiation because these cells are not easily replaced if they die from misfolded, mutated, or otherwise inefficient proteins[1]. Through QC autophagy, damaged cytosolic proteins and organelles are degraded and recycled for production of new, more efficient proteins and organelles. Mitochondria are a primary target for QC autophagy because they are damaged more quickly from internal sources of reactive oxygen species than other organelles[11].

2. Regulation of Autophagy

Activation of the metabolic state sensor AMPK by AMP at low energy levels inhibits mTOR to upregulate autophagy (Fig. 1). When AMP levels rise from consumption of ATP, AMPK phosphorylates mTOR, a protein kinase that phosphorylates ULK1/2 and ATG13. mTOR has two subunits mTORC1 and mTORC2, although only mTORC1 directly inhibits autophagy along with protein synthesis and mitosis [12]. The mechanism for determining whether to regulate autophagy, protein synthesis or mitosis is not known. mTORC1 inhibits the ULK1/2-Atg13 complex through phosphorylation. When mTORC1 is inactive,

dephosphorylation of ULK1/2 activates its kinase activity, phosphorylating ULK1/2, Atg13 and FIP200 [13]. Active ULK1/2-Atg13 complex is localized to the isolation membrane, aiding in vesicle nucleation [14] (Fig. 2). The isolation membrane is an unusual double layered phospholipid bilayer whose origin from mitochondrial [15] or endoplasmic reticular [16] lipids is under investigation. The fungal metabolite rapamycin is a useful drug that directly inhibits mTORC1 to activate autophagy [17]. Rapamycin is used clinically as an immunosuppressant but current research with this drug uses it as a direct, selective inhibitor of mTORC1.

Intracellular signals that inhibit mTOR activity include inadequate energy supply and protein. When the amount of available energy reaches a certain threshold, AMPK is activated. The kinase activity of AMPK phosphorylates TSC1/2, a point of convergence for multiple autophagy regulators [18]. One intracellular protein that regulates mTOR activity is the tumor suppressor p53. Depending on where it resides in the cell, p53 can either stimulate or inhibit the process of autophagy [19]. Under conditions of cellular stress, nuclear p53 activates transcription of autophagy-inducing genes, sestrin2 and dram [20]. Sestrin2 and dram induce autophagy through stimulation of AMPK. Conversely, without stress Mdm2 ubiquitinates p53, signaling p53 nuclear export [21]. Cytosolic p53 inhibits autophagy through direct inactivation of AMPK. Cytosolic p53 inhibits phosphorylation of AMPK, reducing its kinase activity [19].

Lithium and amino acids regulate autophagy through mTOR independent pathways. Lithium inhibits the activity of inositol monophosphatase (IMPase). Without the activity of IMPase, the levels of myo-1,4,5-triphosphate (IP_3) decrease in the cell. A decrease in IP_3 induces autophagy, independent of mTOR. The specific mechanism for IP_3 regulation of autophagy is still unknown [22]. Certain amino acids that are released when autophagy digests proteins (leucine, phenylalanine, alanine, methionine and glutamine) inhibit autophagy are negative feedback regulators of autophagy through the PI3K-III complex (Fig. 1). Amino acid inhibition of the PI3K-III complex stops vesicle nucleation, downstream of mTOR. Conversely, low levels of these amino acids induce autophagy through PI3K-III activation [9]

3. Mitochondria-specific autophagy

Several forms of specific autophagy have been discovered (pexophagy, mitophagy, and cytoplasm-to-vacuole targeting) [23]. Mitophagy, a form of quality control autophagy, is especially important in neurons due to their dependence on oxidative phosphorylation [24]. If damaged mitochondria are not degraded, their increased ROS production can damage the cell. Mitophagy has a unique regulatory system delineated from the mitochondrial dysfunction in Parkinson disease (PD). Two proteins associated with familial PD, PINK1 and Parkin appear to function in mitophagy [25]. As studied in cells without PINK1 such as human embryonic kidney, fibroblasts or HeLa cells, introduction of PINK1 stimulates mitophagy independent of mitochondrial membrane potential. But depolarizing the mitochondria with CCCP increases mitophagy. Normally, the kinase PINK1 is constitutively recruited to mitochondria and degraded (Fig. 3) [26]. To tag a depolarized mitochondrion for mitophagy, (1) degradation is inhibited and PINK1 accumulates. (2) The kinase activity of PINK1 on damaged mitochondria is necessary for recruitment of Parkin, an E3 ubiquitin ligase. (3) Binding to PINK1 activates the VDAC1 specific ubiquitin ligase activity of Parkin [27].

Localization of ubiquitinated mitochondria in aggresomes surrounding the nucleus allows for more efficient, selective sequestering and degradation (Fig. 3). (4) Following Parkin activation, HDAC6 is essential for aggregation of mitochondria via an ubiquitin binding zinc

finger [28]. HDAC6 linked mitochondria are transported to microtubule organizing centers by dynein motors. (5) There, p62 is bound to mitochondria through the ubiquitin tags [29]. p62 also contains an LC3B binding domain, linking the mitochondrial aggregate to the autophagosomal isolation membrane. Simultaneously, ubiquitin bound HDAC6 deacetylates cortactin, to activate its actin remodeling function. Further maturation of autophagosomes allows fusion with lysosomes in a process dependent on an F-actin network [7].

4. Autophagy in Aging: cause or insufficient protection?

Autophagy also plays a role in aging. A popular hypothesis is that aging in humans is a cumulative process starting years, even decades before any symptoms arise[30]. Aging may begin when the rate of damage exceeds the rate of continual repair and turnover. Alternatively, aging could be a last step in epigenetic development [31], which could also pathologically accelerate autophagy. To distinguish these opposite hypotheses, two criteria need to be observed. First, is there a buildup of undigested waste that can be removed by stimulation of autophagy? Or second, if autophagy is overactive, does inhibition of autophagy rescue cell, organ and organism viability. Both of these are best determined by rate measures rather than measures at a single time point.

The best established intervention to delay aging and extend life-span is caloric restriction [32]. As applied to the above hypotheses, does caloric restriction stimulate an aging-protective autophagy or inhibit an overactive anti-aging autophagy? Caloric restriction causes an increase in autophagic proteolysis, leading to a faster turnover of macromolecules and organelles in aging mouse kidney [33]. In studies of protein degradation in rat liver with age, caloric restriction first stimulates autophagy in middle-age, and then delays the onset of age-related decreases in autophagic degradation [34]. The administration of caloric restriction from an early age reduced age-related declines in mouse autophagic sensitivity of hepatocytes to insulin and glucagon[35]. The enhanced activity of autophagy in caloric restriction suggests an aging-protective role of autophagy. Even so, until the effects of autophagic inhibition on caloric restriction have been determined in mammals, a conclusion cannot be made of whether autophagy is necessary for life-span extension in mammals much less neurons. Interestingly, a 24–48 hr. fast was just reported to increase brain neuron autophagy in mice transgenic for GFP-LC3 expression[36]

Mice fed rapamycin continuously late in life had significantly extended life-span compared to controls[37], possibly through inhibition of mTOR and activation of autophagy[38]. This study only looked at survival of rapamycin-fed mice, without examination of levels of specific organ autophagy. Only in yeast has essential knockdown of Atg1 or Atg7, but not Atg11 inhibited life-span extension by rapamycin treatment[39], suggesting action of autophagy.

C. elegans with life-span extending mutations in either the insulin-like signaling pathway, feeding genes, genes in the mitochondrial electron transport chain, or the inhibition of protein translation produce mitochondria with less polarized membrane potentials[40]. The same extension of life-span can be seen in treatment with mitochondrial electron transport chain uncouplers. The effect of uncoupler treatment is dose-dependent, becoming toxic if too high[40]. The mechanism by which a less polarized mitochondrial membrane potential extends lifespan is unknown, but could be due to a lower rate of ROS production or increased mitophagy at less polarized membrane potential [41]. Mitophagy is induced through mitochondrial membrane depolarization [26]. The correlation in *C. elegans* of life-span extension with an optimum extent of inhibition of mitochondrial electron transport [42] could be the point at which mitochondrial efficiency is balanced with optimum turnover by mitophagy, but this has not been established in any organism.

Another aspect of aging is the buildup of protein and lipid aggregates as lipofuscins in lysosomes that inhibit lysosomal degradation in mitophagy [11]. Enzymes continue to be delivered to lysosomes containing lipofuscin, removing them from availability for autophagosomal degradation with functional lysosomes. The buildup of lipofuscins is a problem mostly seen in long-lived cells, such as neurons and continues to accumulate until it inhibits cellular processes[43].

As reviewed by Moreira et al., damaged mitochondria produce ATP at reduced rates [44]. A decrease in ATP levels by inhibition of complex I with rotenone initially increases the rate of autophagy in human neuroblastoma cells [45]. Further depletion of ATP could inhibit autophagy during sequestering and lysosomal degradation[46]. ATP is needed for the ubiquitin-like conjugation systems to elongate the autophagosomal membrane [47]. Without the ubiquitin-like conjugation systems, the sequestering step cannot form autophagosomes, stopping the entire process. Lysosomal hydrolase activity is positively correlated with the amount of available ATP present[48]. Without ATP the lysosomal proton pumps cannot create the acidic environment necessary for degradation by lysosomal proteases, lipases and nucleases.

In *Drosophila*, over-expression of Atg8 (LC3 homologue) to induce autophagy extends lifespan by 50% and increases resistance to ROS cytotoxicity [49]. A complementary approach in mouse neurons of autophagic inhibition through knockdown of Atg5 causes accumulation of polyubiquitinated proteins and increased cell death [50]. If the absence of autophagy causes aging, then the proper function of autophagy is likely to be protective. Therefore, a gradual decline or insufficient autophagy could be a major contributor to aging.

5. Autophagy in Alzheimer's disease: pathologic or failed protective response?

Autophagosomes accumulate in AD cortex, compared to non-demented controls[51]. The accumulation of autophagosomes in AD could be due to a combined stimulation of induction of autophagy or a residual slow rate of autophagosome formation together with failure to complete sufficient lysosomal fusion and digestion. For evidence related to induction, protein and mRNA expression of beclin 1, a function necessary for vesicle nucleation (Fig. 1) was examined. The protein and mRNA of beclin 1 is decreased in AD cortex compared to controls [52], suggesting an inhibition of autophagy in AD. Furthermore, a beclin 1 (+/-) knock-down crossed into an AD mouse model promoted A β accumulation which was reversed by genetic (lentivirus) restoration of beclin [52], additionally suggesting that autophagy of A β aggregates may be inhibited in AD. In a complimentary approach, two AD mouse models were treated with rapamycin for 10–13 weeks to determine the role of autophagy in AD pathology and memory [53, 54]. Rapamycin promoted autophagy to cause a decrease in A β accumulation in pyramidal neurons and delayed memory deficits, while inhibition of autophagy reversed the effect. Therefore, stimulation of autophagy could be therapeutic for AD. Although enhanced induction of autophagy rescues AD mouse models from AD-like pathology, the conflicting observations of abundant autophagosomes and decreased beclin1 at single post-mortem time points suggests that there may be more than one deficit in autophagy in the AD brain.

At the end of autophagy, lysosomes need to properly fuse with and degrade the contents of the autophagosome. Since the lysosomal protease cathepsin D also accumulates in AD, compared to age-matched non-demented control brains[55], lysosome biosynthesis appears more than adequate, but lysosomal function may be inhibited in AD brain. Another approach determines the effect of familial AD mutations on lysosomal function and the late stages of autophagy. Familial AD presenilin 1 mutations inhibit the acidification of lysosomes in

human fibroblasts from AD patients [56]. Autophagosomes accumulated in this model, presumably due to failure of autophagosome protein degradation after fusion with lysosomes. This defective lysosome model could explain the increase in autophagosomes seen in AD neurons, even if autophagosome formation is decreased.

In normal A β production, endocytosis brings the APP membrane protein into the early endosome, where A β is produced. The A β is then recycled back to the plasma membrane [57]. Autophagosomes have also been found to contain the proteins necessary for A β production [58]. In properly functioning autophagy, the A β produced is degraded in lysosomes.

Together, steps in between autophagosome formation and degradation, especially lysosome fusion need to be delineated in AD brain (Fig. 2). Further measures of the rate of autophagy at these later steps in a mouse model of AD may also clarify the details of the process.

6. Reactive oxidizing species (ROS) as an initiator or consequence of autophagy

Accumulation of oxidative damage to macromolecules is seen as neurons age [59]. At a certain point, the level of oxidative stress will inhibit cellular processes, including autophagy and possibly even cause cell death. However, ROS damage to mitochondria depolarizes the mitochondrial membrane potential to initiate mitophagy, and possible cell survival. With age, ROS reacting with mitochondrial fatty acids produces lipofuscin, an indigestible lipid aggregate [60]. But inhibition of ROS production has proven ineffective in treatment of age-related diseases, perhaps because small amounts of ROS are needed as cell signals [61]. In autophagy induced by starvation, ROS signals are required to inhibit Atg4 [62]. The Atg4 protease cleaves LC3-I, allowing lipidation as LC3-II, followed by ubiquitination of Atg5 and 12 and an Atg4-dependent recycling of the LC3-II back to LC3-I as the lipid is cleaved. PI3K-III produces ROS upon activation of autophagosome formation, inhibiting Atg4 after LC3-II formation, allowing autophagosome formation by inhibiting the lipid removal activity of Atg4. However, higher concentrations of ROS were necessary for induction of autophagy by A β in neuroblastoma cells [63].

In AD, lysosomal degradation appears inhibited, causing accumulation of autophagosomes in neurons. These autophagosomes continue to produce A β [57]. If these autophagosomes rupture, the A β released will damage mitochondria, enforcing a vicious cycle of damaged mitochondria in neurons, increased induction of mitophagy to form more autophagosomes. In AD, A β accumulates in mitochondrial membranes, disrupting the electron transport chain and increasing ROS production leading to more mitochondrial protein oxidation [64]. In a mouse model of AD that typically shows memory loss, the overexpression of mitochondrial manganese superoxide dismutase (MnSOD) prevented memory loss, suggesting that protection from mitochondrial ROS damage is critical to reversing the effects of A β accumulation in mitochondria [65].

7. Future directions

There are still many gaps that need to be filled in the current model of autophagy, beginning with identification of more mammalian homologues of the yeast Atg genes. Increased complexity of mammalian organisms requires different interactions between Atg proteins. Uncovering the role of autophagy in healthy cells might allow greater access to QC autophagy in therapy of neurodegenerative diseases, as well as general aging.

Treatments that inhibit mTOR activity are effective in increasing autophagosome creation; although there are situations where autophagy induction is ineffective. The possible defect of autophagosomal degradation in AD is a new area of study with no treatments known. The treatment of Beclin 1 deficiency in AD may be insufficient without the combined treatment to stimulate exocytosis of lipofuscin or promote its degradation and vice versa.

Although a large portion of the proteins and steps necessary for mitophagy are known in yeast, there are still gaps in the understanding of this process in mammalian cells. Methods of manipulating mitophagy without harming the cell might be useful for anti-aging therapy with consequent impact on AD. Although mutated Parkin is a part of Parkinson's disease pathology, the normal form is necessary for normal cell function. Kawajiri et al. showed that transfecting cells with PINK1 and Parkin expressing vectors can induce mitophagy[25]. This same principle could be applied to mitophagy using lentiviral vectors containing PINK1 and Parkin for use in anti-aging or anti-AD therapy. The use of lentiviral vectors may allow for therapy to be administered to specific areas without affecting the surrounding tissues.

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Fig 1.

Regulation of autophagy is regulated by both extracellular and intracellular signals. Low AMP/ATP, growth factor deprivation or nutrient restriction inhibit mTOR activity to promote autophagy, although amino acids and lithium act independently of mTOR. The inhibition of mTOR induces autophagy, protein synthesis and mitosis. Hexagon = cellular processes regulated by mTOR.

Fig 2.
The induction of autophagy uses step-wise activation of several protein complexes (ULK1/2, PI3K-III, LC3-II-PE, and Atg12-Atg5-Atg16) resulting in autophagosome formation. See text for detail about highlighted enzymes.

Fig 3.

Initiation of mitophagy requires PINK1, Parkin, HDAC6, p62, and cortactin in addition to many of the autophagy proteins in Fig. 2. In 5, the dashed lines indicate the possible completion of the autophagosomal membrane. Figure adapted from [66].

Table 1

Atg protein homology from yeast to humans

yeast	human	Function	Step in autophagy
Atg1	ULK1 ULK2	Kinase in ULK-Atg13-FIP200 complex, (autophagy induction) [67]	Nucleation
Atg2	Atg2		
Atg3	Atg3	E2 activity in LC3 processing [68]	Elongation
Atg4	Atg4A Atg4B Atg4C Atg4D	LC3 cleavage to allow and remove lipid group [62]	Elongation
Atg5	Atg5	Part of Atg12-Atg5-Atg16 complex [69]	Elongation
Atg6	Beclin-1	PI3K-III complex [9]	Nucleation
Atg7	Atg7	E1 activity in both LC3 and Atg12-Atg5-Atg16 pathways [69]	Elongation
Atg8	GABARAP GABARAPL1 GABARAPL2 GABARAPL3		Elongation Elongation Elongation Elongation
	MAP1LC3A	Expressed in heart, brain, liver, skeletal muscle, and testis [6]	Elongation
	MAP1LC3B fusion fusion fusion	Expressed in heart, brain, skeletal muscle, and testis; least abundant in liver [6]	Elongation
	MAP1LC3C	Expressed in placenta, lung, and ovary, very low expression in other areas [6]	Elongation
Atg9	Atg9A Atg9B		
Atg10	Atg10	E2 activity in Atg12-Atg5-Atg16 pathway [69]	Elongation
Atg11	--		
Atg12	Atg12	Part of Atg12-Atg5-Atg16 complex [69]	Elongation
Atg13	Atg13	ULK-Atg13-FIP200 complex, mediates ULK1/2-FIP200 interactions [67]	Nucleation
Atg14	--		
Atg15	--		
Atg16	Atg16L1 Atg16L2	Part of Atg12-Atg5-Atg16 complex [69]	Elongation
Atg17	FIP200	ULK-Atg13-FIP200 complex [67]	Nucleation
Atg18	Atg18A Atg18B		
Atg19	--		
Atg20	--		
Atg21	--		
Atg22	--		
Atg23	--		

yeast	human	Function	Step in autophagy
Atg24	Atg24A Atg24B		
Atg25	--		
Atg26	--		
Atg27	--		
Atg28	--		
Atg29	--		
Vps15	Vps15		
Vps34	Vps34		