Autophagy Failure in Alzheimer's Disease – Locating the Primary Defect

Ralph A. Nixon[1,2,3,*] and **Dun-Sheng Yang**[1,2]

[1]Center for Dementia Research, Nathan Kline Institute, Orangeburg, NY

[2]Department of Psychiatry, New York University Langone Medical Center, New York, NY

[3]Department of Cell Biology, New York University Langone Medical Center, New York, NY

Abstract

Autophagy, the major degradative pathway for organelles and long-lived proteins, is essential for the survival of neurons. Mounting evidence has implicated defective autophagy in the pathogenesis of several major neurodegenerative diseases, particularly Alzheimer's disease (AD). A continuum of abnormalities of the lysosomal system has been identified in neurons of the AD brain, including pathological endocytic pathway responses at the very earliest disease stage and a progressive disruption of autophagy leading to the massive buildup of incompletely digested substrates within dystrophic axons and dendrites. In this review, we examine research on autophagy in AD and evaluate evidence addressing the specific step or steps along the autophagy pathway that may be defective. Current evidence strongly points to disruption of substrate proteolysis within autolysosomes for the principal mechanism underlying autophagy failure in AD. In the most common form of familial early onset AD, mutant presenilin 1 disrupts autophagy directly by impeding lysosomal proteolysis while, in other forms of AD, autophagy impairments may involve different genetic or environmental factors. Attempts to restore more normal lysosomal proteolysis and autophagy efficiency in mouse models of AD pathology have yielded promising therapeutic effects on neuronal function and cognitive performance, demonstrating the relevance of autophagy failure to the pathogenesis of AD and the potential of autophagy modulation as a therapeutic strategy.

Introduction

Alois Alzheimer's discovery of neurofibrillary tangles, and their presence together with senile plaques in the brain of a patient with progressive dementia, established the two neuropathologic features that still define Alzheimer's disease (AD). Coinciding with these seminal studies were more detailed descriptions of senile plaques by Marinesco, Fischer, and even Ramon y Cajal (Garcia-Marin et al., 2007; Goedert, 2009), which emphasized the widespread incidence of gross focal swellings of axons and possibly dendrites, termed dystrophic neurites, that were particularly profuse within senile plaques but were also seen throughout affected regions of the parenchyma. Although dystrophic neurites were originally detected by Alzheimer from their argyrophilia, reflecting pathologically hyperphosphorylated cytoskeletal proteins, dystrophic swellings in the AD brain were

[*]Please address correspondence to: Ralph A. Nixon, PhD, MD, Nathan Kline Institute, 140 Old Orangeburg Rd, Orangeburg, NY 10962, nixon@nki.rfmh.org.

subsequently shown to be filled mainly with autophagic vacuoles-vesicular compartments of the autophagic-lysosomal pathway (Nixon et al., 2005). In well-preserved biopsied AD neocortex, ultrastructural and immunogold labeling studies have distinguished not only dense lysosomes (Terry et al., 1964) but also various types of autophagic vacuoles (AVs) including autophagosomes, amphisomes, multilamellar bodies, and autolysosomes, representing "intermediate" stages in the progression of autophagy (Nixon et al., 2005). These observations suggested that the normally efficient autophagic process in neurons is stalled in AD. In recent studies, investigators have begun to pinpoint the site or sites at which autophagy may be disrupted and explore the possibility that modulating specific steps in this process could have a therapeutic impact on pathology and function in AD mouse models. In this review, we consider evidence indicating that neuronal autophagy is defective in AD and evaluate data addressing mechanisms underlying this defect and possible implications for the pathogenesis and therapy of AD.

The stages of autophagy

The term autophagy refers to all processes in which components of the cell are degraded in lysosomes/vacuoles and recycled (Klionsky et al., 2010). Based on how substrates are delivered to the lysosomal compartment, autophagy is classified into three general subtypes in most mammalian cells: chaperone-mediated autophagy (CMA), microautophagy and macroautophagy (Cuervo, 2004; Mizushima et al., 2008). In CMA (Cuervo, 2010), cytosolic proteins containing a KFERQ motif are selectively targeted to the lysosomal lumen for degradation. In a second process called microautophagy, small quantities of cytoplasm are non-selectively introduced into lysosomes when the lysosomal membrane invaginates and pinches off small vesicles for digestion within the lumen. Finally, a third pathway, macroautophagy, conserved from yeast to mammals, mediates large-scale degradation of cytoplasmic constituents including organelles (He and Klionsky, 2009). Macroautophagy, referred to by the general term autophagy in this review, is considered the greatest contributor to the overall autophagy rate under most conditions. This process is regulated by signaling cascades mediated through the liver kinase B1 (LKB1)/AMP-activated protein kinase (AMPK) or the Class I phosphatidylinositol 3-kinase (PI3K)/Akt pathways which converge on the mammalian target of rapamycin (mTOR) kinase through TSC/Rheb (tuberous sclerosis complex/Ras homolog enriched in brain) (Hay and Sonenberg, 2004; He and Klionsky, 2009). The inhibition of mTOR sets in motion a sequence of events coordinated by complexes of autophagy-related (Atg) proteins that initiate the formation of the autophagosome (Diaz-Troya et al., 2008; Levine and Kroemer, 2008).

Autophagy begins when an 'isolation membrane' is created from a pre-autophagosomal structure (PAS) or phagophore, such as the ER-derived cup-shaped omegasome, and sequesters a region of cytoplasm to form a double-membrane-limited autophagosome (Axe et al., 2008; Geng et al., 2010; Hailey et al., 2010; Hayashi-Nishino et al., 2009; Itakura and Mizushima, 2010; Ravikumar et al., 2010; Tian et al., 2010; Xie and Klionsky, 2007). The outer membrane of the autophagosome then fuses with a lysosome or a late endosome to form either an autolysosome or an amphisome, respectively, thereby initiating the digestion of sequestered material by a range of acidic hydrolases (Eskelinen, 2005; Fader and Colombo, 2009; Gordon and Seglen, 1988; Liou et al., 1997; Noda et al., 2009). Acidification of autolysosomes by vacuolar [H+] ATPase (v-ATPase), a proton pump assembled on the lysosome membrane, is crucial for activating cathepsins and effecting proteolysis of substrates (Yoshimori et al., 1991). The completion of substrate digestion within autolysosomes ultimately yields lysosomes, which are smaller, less dense vesicles containing mainly lysosomal hydrolases (Figure 1) (Nixon, 2007; Yu et al., 2010). While the term "maturation" is often used to describe the fusion between autophagosomes and lysosomes/late endosomes (Noda et al., 2009), maturation is a continuous process that

includes completion of substrate degradation within autolysosomes and the restoration of lysosomes. The functional relationship among different stages of autophagy is underscored by the recent finding that the beginning of this process (i.e., autophagosome formation) and its end (the reformation of lysosomes) are both regulated by mTOR (Yu et al., 2010). In addition to referring to the specific subtypes of autophagy-related vesicular structures, we will also use the term autophagic vacuoles (AVs) as the general term for autophagy-related vesicular structures that include autophagosomes, amphisomes, multilamellar bodies, and autolysosomes.

Since its very early descriptions, autophagy has been defined as the lysosomal *digestion* of a cell's own cytoplasmic material, and not simply as the sequestration of these components. Appreciation of this notion has become particularly critical to understanding how autophagy may be involved in disease states because impairments of any of the steps in autophagy may result in a diminished turnover of specific autophagy substrates. Identifying which step along the autophagy pathway may be defective in a given neurodegenerative disease, therefore, requires an evaluation of autophagosome formation, autophagosome clearance by lysosomes, and autophagic flux—the rate at which a given substrate cycles through the entire autophagy process. Autophagic flux reflects a dynamic balance between the rates of substrate sequestration and degradation (Figure 2) (Chu, 2006;Wong and Cuervo, 2010).

Autophagy pathology in the AD brain is extensive

Abnormalities of lysosomal system function in the AD brain range from the earliest known disease-specific pathology in the disease, which involves the endocytic pathway (Nixon and Cataldo, 2006), to the most abundant pathology in the AD brain involving the autophagic pathway, which is the main focus of this review. The autophagy pathology of AD, including accumulated AVs of all types and the numbers of dystrophic neurites containing these AVs, is uniquely extensive when compared to that in other aging-related neurodegenerative diseases (Nixon and Cataldo, 2006; Nixon et al., 2005). The near complete replacement of normal cytoplasmic contents by AVs in dystrophic neurites and the increased frequency of AVs in less extensively affected neurites (Figure 3), together with the uniquely high number of dystrophic neurites in senile plaques and elsewhere throughout the AD brain, represents an enormous "burden" of undigested or partially digested proteins. This burden of waste proteins is comparable to that seen in certain primary lysosome storage disorders (LSDs), which are associated with severe neurodegeneration in early life (Nixon et al., 2008). Interestingly, neurofibrillary tangles and increased amyloidogenic processing of amyloid precursor protein (APP) as well as neuritic dystrophy, have been identified in certain primary LSDs as well as AD and potentially represent clues about common pathogenic mechanisms (see below). Autophagy-related pathology, including lesser degrees of AV "storage" in neurons, has also been increasingly recognized in other late-onset neurodegenerative diseases (Anglade et al., 1997; Liberski et al., 1995; Rudnicki et al., 2008; Yue et al., 2002; Zhou et al., 1998), although the severity and extent of the neuritic dystrophy in AD (Figure 3) (Masliah et al., 1993; Nixon et al., 2005; Schmidt et al., 1994; Suzuki and Terry, 1967) distinguishes it from these other aging-related neurodegenerative diseases (Benzing et al., 1993).

It is noteworthy that the composition of organelles within dystrophic swellings in the AD brain also differs from that seen in the many other disorders characterized by neuroaxonal dystrophy. In these other conditions vesicles and cytoskeletal elements of all types are relatively abundant, resembling the pattern seen after axonal transport is focally interrupted by nerve ligation or toxic agents (Griffin and Watson, 1988; Schmidt and Plurad, 1985; Wirtschafter et al., 1977), suggesting a general failure of the axonal transport system. By contrast, a more selective transport deficit affecting mainly autophagy-related compartments

is seen in mouse and cell models of AD pathology. Indeed, recent live-imaging studies of cortical neurons from our laboratory demonstrate that inhibiting lysosomal proteolysis, as believed to occur in AD neurons, selectively disrupts the axonal transport of autophagy-related compartments, causing an AD-like axonal dystrophy characterized by selective AV accumulation (Sooyeon Lee and Ralph Nixon, unpublished results). Therefore, a general disruption of axonal transport, as often accompanies neurodegeneration, cannot account for the particular pattern of organelle accumulation within dystrophic neurites in AD. Defective autophagic lysosomal proteolysis, as discussed below, is likely to contribute to the development of this pathology.

Autophagy in normal neurons is constituently active, inducible and highly efficient

Autophagic vacuoles are uncommon in neurons of the healthy brain (Cota et al., 2006; Mizushima et al., 2008; Mizushima et al., 2004; Nixon et al., 2005). These observations initially led some investigators to assume that autophagy is relatively inactive in neurons unless it is induced, although this now seems unlikely. Autophagosomes are "intermediates" in a multi-step pathway and their presence in cells depends on both their rate of formation and rate of clearance by lysosomal degradation (Figure 2). As long as their clearance matches or exceeds production, autophagosomes or other AV "intermediates" may be rarely detected, even though autophagy flux—the net rate of formation and clearance of autophagosomes—could be very rapid (Boland et al., 2008; Chu, 2006). Illustrative of this point is the observation in cultured primary neurons that blocking autophagosome clearance by inhibiting cathepsins causes rapid AV accumulation without altering autophagy induction (Boland et al., 2008). These observations indicate that autophagy is constitutively active in neurons and that efficient clearance of autophagosomes keeps their numbers low. Consistent with this concept, deletion of genes essential for autophagy results in the accumulation of protein aggregates and neuronal cell death implying that constitutive autophagy is essential for both normal protein turnover and for neuron survival (Hara et al., 2006; Komatsu et al., 2006).

Recent studies suggest that both autophagic flux and the magnitude of the autophagy induction in response to classical autophagy inducers may vary in different types of neurons. In primary cortical neurons (Boland et al., 2008), LC3 and AVs accumulate when lysosomal proteolysis is inhibited but this accumulation is dramatically increased when autophagy is also induced by rapamycin or starvation. By contrast, primary striatal neurons exhibit a significant level of constitutive autophagic flux but little additional response to rapamycin (Tsvetkov et al., 2010; Tsvetkov et al., 2009). Based on these observations, Tsvetkov et al. have suggested that neurons may be poorly responsive to rapamycin and related conventional autophagy inducers (Tsvetkov et al., 2010). Other investigators, however, have shown that neurons form autophagosomes and, more importantly, accelerate autophagic flux (Rubinsztein and Nixon, 2010) after rapamycin (Alirezaei et al., 2008; Boland et al., 2008; Crews et al., 2010; Rose et al., 2010) and starvation (Du et al., 2009). At least a partial explanation for the different conclusions may lie in observations that the clearance of LC3-positive autophagosomes in cortical neurons is very efficient even after strong induction (Boland et al., 2008). Neurons exposed to rapamycin or starvation alone accumulate mainly late AVs (i.e., autolysosomes) containing extensively digested substrates and therefore exhibit only modest elevations of LC3. However, when autophagic flux is measured in the presence of the cathepsin inhibitor leupeptin, LC3 compartments accumulate robustly, showing that induction of autophagy is, indeed, quite robust (Boland et al., 2008). It is likely, however, that autophagy is regulated differently in neuronal subtypes, glial cells and non-neuronal cells and future analyses of this issue, which require assessments of autophagy

with and without lysosomal inhibitors, will be instructive to elucidate canonical and non-canonical autophagy pathways and their relative activities.

Autophagy in AD is principally defective at the stage of autolysosomal proteolysis

Converging lines of evidence indicate that a disruption of the proteolytic clearance of AVs by lysosomes is the principal basis for the massive neuronal accumulation of AVs in the AD brain. Even when autophagy is strongly induced in primary cortical neurons by rapamycin, autophagosomes can rapidly mature to autolysosomes and then to electron-translucent lysosomes without a major buildup of "intermediate" AVs (Boland et al., 2008). By contrast, the AVs accumulating in the AD brain are electron-dense autolysosomes and autophagosomes filled with undigested or partially digested substrates (Nixon et al., 2005). These substrate-filled intermediates accumulate in AD neurons even though the expression of lysosomal hydrolases is strongly upregulated (Cataldo et al., 1995; Cataldo et al., 1996) and the abundant lysosomes in dystrophic neurites are able to fuse with accumulating AVs. A similar pattern of AV accumulation and neuritic dystrophy is observed when lysosomal degradation is inhibited by deleting one or more cathepsins (Felbor et al., 2002; Koike et al., 2000; Koike et al., 2005) or by using cysteine protease inhibitors or general lysosomal enzyme inhibitors *in vivo* (Bednarski et al., 1997; Boland et al., 2008; Ivy et al., 1989; Ivy et al., 1984; Takeuchi and Takeuchi, 2001; Yang et al., 2008a). In cathepsin L deficient cells, for example, electron-dense autolysosomes accumulate even though autophagy is not induced and the formation of autophagosomes and fusion with lysosomes are minimally affected (Dennemarker et al., 2010), consistent with the results of Boland et al. (2008) in neurons.

A critical role for lysosomal proteolytic failure in the development of AD-related neurodegeneration is further supported by studies of primary LSDs – a group of more than 50 genetic disorders caused by defective lysosomal and non-lysosomal proteins, resulting in accumulation of autophagic and endosomal substrates. While the effects resulting from the deficiency of a specific lysosomal protein in a given LSD are ubiquitous, some LSDs, such as GM1 and GM2 gangliosidoses, Niemann Pick type C disease (NPC) and neuronal ceroid-lipofuscinosis (NCL), are associated with prominent nervous system degeneration each involving disruption of the internal environment of the lysosome by dissimilar lysosomal factors (Jeyakumar et al., 2005; Walkley, 1998; Walkley, 2009). Moreover, similarities are being identified between the cellular pathologies of AD and of certain LSDs (Chevrier et al., 2010; Eckhardt, 2010; Fukuda et al., 2006; Koike et al., 2005; Settembre et al., 2008). For instance, neurofibrillary tangles, a diagnostic hallmark of AD, are seen in NPC and mucopolysaccharidosis type IIB (Ohmi et al., 2009) and in a mouse model of this disease (Ryazantsev et al., 2007), in addition to the profuse AV accumulations seen also in AD brains. In NPC, which arises from a defect in endosomal trafficking of cholesterol and the accumulation of unesterified cholesterol in late endosomes/lysosomes, additional features overlap with those in AD, including highly disease-selective abnormalities of endosomes, elevated levels of the beta-site APP-cleaving enzyme (BACE), and BACE-cleaved carboxyl-terminal fragments of APP (βCTF), and mild deposition of the amyloid-β peptide (Aβ). Allele-selective influences of apolipoprotein E (APOE) genotype on pathology are also common to both NPC and AD (Nixon, 2004).

AD genetics directly support the pathogenic importance of lysosomal proteolytic failure in AD

a. Role of presenilin 1 (PS1) in autophagic-lysosomal proteolysis

Presenilin 1 (PS1) mutations are the most common cause of early-onset familial AD (FAD) (Sherrington et al., 1995). PS1, a ubiquitous transmembrane protein, has diverse biological roles in cell adhesion, apoptosis, neurite outgrowth, calcium homeostasis and synaptic plasticity (Shen and Kelleher, 2007). Most of these roles involve PS1 as the catalytic subunit of the γ-secretase complex that mediates intramembrane cleavage of over 20 known substrates, including APP. However, PS1 also has γ-secretase-independent roles, which notably include a critical role in lysosome acidification that is essential for the activation of lysosomal proteases during autophagy. A failure to degrade autophagy substrates and clear AVs in PS1-null blastocysts and neurons of mice conditionally depleted of PS1 was recently established on the basis of abnormally elevated levels of autophagy substrates (Esselens et al., 2004; Wilson et al., 2004), inhibited macroautophagic turnover of radiolabeled proteins, impaired cathepsin maturation, and reduced cathepsin-specific activities. All of these effects of PS1 deletion are the outcomes of the loss of a specific role of PS1 in the acidification of autolysosomes/lysosomes. PS1 deficiency is associated with the failure of the V0a1 subunit of v-ATPase to become N-glycosylated in the ER, rendering it susceptible to increased degradation before it can be delivered to autolysosomes/lysosomes at levels sufficient to support normal lysosomal acidification (Lee et al., 2010).

Of particular clinical importance, fibroblasts from patients with FAD caused by PS1 mutations also exhibit markedly defective lysosome acidification and autolysosome maturation, similar in mechanism to that seen in PS1-null cells (Lee et al., 2010). Given that autophagy is essential for neuron survival (Mizushima et al., 2008), the marked autophagy impairment in PS1-null or PS-FAD fibroblasts can account for observations that PS1 mutations promote cell death in injured neurons (Chui et al., 1999; Guo et al., 1997). This defect of lysosomal function leading to inefficient protein/peptide clearance can also provide a basis for earlier observations that PS1 mutations in the brains of patients with PS-FAD or mouse models potentiate autophagic-lysosomal, amyloid, and tau pathologies as well as accelerate neuronal cell death (Cataldo et al., 2004).

b. Amyloid Precursor Protein (APP) and ApoE

Additional AD-related genes and environmental risk factors disrupt lysosomal system function and also support a critical role of lysosomal failure in the progression of AD. Severe autophagy neuropathology develops in all forms of AD and also in mouse models of AD where only FAD-related mutant forms of APP are overexpressed, although the onset is often later than that in corresponding disease variants involving PS1 mutations. For example, autophagiclysosomal pathology in TgCRND8 mice overexpressing human APP with two FAD mutations (Chishti et al., 2001; Yang et al., 2008b; Yang et al., 2011) is nearly as florid as that seen in the PS/APP mouse model of AD (Cataldo et al., 2004; Yu et al., 2005), indicating that mutant APP overexpression alone can lead to autophagic-lysosomal pathology. Hippocampal and cortical neurons in these mice develop strikingly enlarged autolysosomes containing incompletely digested materials, including membranous structures and a minor lipopigment component, resembling the ceroid-containing AVs seen in the brain when one or more cathepsins are deleted (Koike et al., 2005) or inactivated pharmacologically (Bednarski et al., 1997; Ivy et al., 1989; Takeuchi and Takeuchi, 2001). Failed autophagic digestion is also evidenced by the accumulation of undigested autophagy substrates including LC3-II, ubiquitinated proteins, and Aβ peptides in AVs and lysosomes isolated from the brains of these mice. Moreover, stimulating lysosomal proteolytic efficiency in the TgCRND8 mice by deleting an endogenous inhibitor of lysosomal cysteine

proteases (cystatin B), rescues the lysosomal pathology, eliminates abnormal autolysosomal accumulation of autophagy substrates including Aβ and ubiquitinated proteins, decreases extracellular amyloid deposition and total brain Aβ40/42 levels, and ameliorates learning and memory deficits (Yang et al., 2011). These observations support the pathogenic significance of autophagic-lysosomal dysfunction in AD and specifically the importance of deficient lysosomal proteolysis. Therapeutic effects have also been reported in another AD model of β-amyloidosis after over-expressing cathepsin B or deleting cystatin C (Mueller-Steiner et al., 2006; Sun et al., 2008). These findings suggest the potential value of targeting impaired lysosomal proteolysis as a therapeutic strategy for AD.

The mechanism by which overexpression of mutant APP may lead to impaired autophagy and neuritic dystrophy, is not well understood. One possible contributor may be the toxic actions of the βCTF of APP on the endosomal-lysosomal system, which is also a possible basis for other AD subtypes, including sporadic AD. The earliest disease-specific pathologic change in sporadic AD—appearing before amyloid is deposited in the neocortex—is the enlargement of Rab5- and Rab7-positive endosomes (Cataldo et al., 2008; Cataldo et al., 2000), which reflects a pathological acceleration of endocytosis (Cataldo et al., 2008; Ginsberg et al., 2010; Grbovic et al., 2003; Jiang et al., 2010). This pattern is specific for AD among the aging-related neurodegenerative diseases studied and is exacerbated by inheritance of the ε4 allele of APOE, the major genetic risk factor for late onset AD (Cataldo et al., 2000). The same pathological endosomal response is also seen in Down's syndrome (trisomy 21), a cause of early onset AD, where it is linked specifically to an extra copy *of App* on the trisomic region of chromosome 21 and is mediated by βCTF (Cataldo et al., 2003; Jiang et al., 2010). The acceleration of endocytosis in cells of individuals with Down syndrome also causes increased protein and lipid accumulation in endosomes and slowed lysosomal degradation of endocytic cargoes (Cataldo et al., 2008). By upregulating endocytosis, elevated dietary cholesterol and over-expression of its receptor ApoE (particularly ApoE4) elevate βCTF levels and also lead to increased delivery of Aβ1-42 to lysosomes (Cossec et al., 2010; Ji et al., 2006). Expression of an APOE ε4 allele, but not the APOE ε3 allele, in a mouse AD model increases levels of intracellular Aβ in lysosomes, altering their function and causing neurodegeneration of hippocampal CA1, entorhinal, and septal neurons (Belinson et al., 2008). Thus, in sporadic AD and under conditions of increased genetic and environmental AD risk, accelerated endocytosis mediated by Rab5 and βCTF seems to be a common pathway in AD leading to increased delivery of substrates into degradative compartments. The reduced efficiency of lysosomal degradation combines with additional effects of oxidative damage (Kurz et al., 2008; Terman and Brunk, 2006) and other toxic factors, including Aβ, to impair lysosomal and autophagy function. Impaired lysosomal degradation, in turn, promotes accumulation of proteins and peptide fragments (e.g., Aβ, tau) that can further accelerate this toxic cascade.

It has been proposed that the low pH of lysosomes accentuates the conversion of ApoE4 to a molten globule, induces reactive intermediates capable of destabilizing cellular membranes leading to lysosomal leakage and apoptosis (Ji et al., 2002; Ji et al., 2006; Mahley and Huang, 2006). Consistent with these findings, strong overexpression of human Aβ42, but not Aβ40, in *Drosophila* neurons induces age-related autophagic-lysosomal dysfunction characterized by autolysosome accumulation and neurotoxicity (Ling et al., 2009). Aβ42-induced neurotoxicity is further enhanced by autophagy activation and is partially rescued by autophagy inhibition. The authors propose that the structural integrity of post-fusion AVs may be compromised in Aβ42 affected neurons, leading to subcellular damage and loss of neuronal integrity in the Aβ42 flies, as originally demonstrated by Glabe, Yang and colleagues in studies of Aβ uptake in neuronal cells. In these cells, internalized Aβ1-42 is slowly degraded and therefore accumulates in lysosomes, which is followed by leakage of

lysosomes enzymes into the cytosol, preceding morphological evidence for cellular toxicity (Glabe, 2001; Yang et al., 1998).

Evidence for altered autophagy induction in AD is conflicting

Evidence supporting additional abnormalities of autophagy at the level of induction or autophagosome formation as a possible basis for autophagy dysfunction in AD is equivocal so far. Although autophagosomes are more numerous in the AD brain and in the PS1/APP mouse model of AD pathology (Yu et al., 2005), this cannot be considered strong support specifically for autophagy induction if AV clearance is also defective as previously discussed. Strong autophagy induction in association with neurodegeneration and autophagic cell death is so far uncommon compared to neurological responses associated with autophagy deficiency (Nixon, 2006; Wong and Cuervo, 2010).

Supporting the possibility that autophagy induction could contribute to AD pathology is recent evidence that the synthesis of many components of the lysosome is upregulated at the transcriptional and translational levels in the AD brain and AD mouse models (Cataldo et al., 1995; Cataldo et al., 1996; Cataldo et al., 2004; Ginsberg et al., 2010; Ginsberg et al., 2000; Mufson et al., 2002; Nixon and Cataldo, 2006). Moreover, Lipinski et al. recently reported that the transcription of factors promoting autophagy is up-regulated in the brains of AD patients, while negative regulators of autophagy as a group are down-regulated (Lipinski et al., 2010). Oxidative stress and production, an early pathological event in AD (Perry et al., 2002; Zhu et al., 2007), contributes to this apparent induction of autophagy by activating type III PI3 kinase (Lipinski et al., 2010). Reduced mTOR kinase activity, reflected in lowered levels of phosphorylated p70S6 kinase, has also been seen in cells treated with Aβ1-42 and in brains of PS1/APP mice, although not all studies are consistent with a stimulatory effect of Aβ on induction. For example, Aβ oligomers stimulated mTOR activity in cultured neurons while promoting cell cycle events associated with later apoptosis (Bhaskar et al., 2009). Moreover, mTOR enzymatic activity and phosphorylated p70S6K levels were also elevated in CHO cells stably transfected with FAD-related mutant APP and in the cortex and hippocampus of 3xTg-AD mice (Caccamo et al., 2010). In these mice, rapamycin administered beginning early in life restored mTOR signaling, decreased Aβ and tau pathology, and ameliorated cognitive deficits. Activated forms of mTOR and downstream mTOR-dependent factors promoting mRNA translation were detected in the AD brain within neurons exhibiting neurofibrillary degeneration, suggesting increased translation of certain transcripts and implying that autophagy may be down-regulated (Li et al., 2005).

In further support for impairment in the early stages of autophagy, levels of the autophagy-related protein beclin-1, a component of the PI3 kinase complex essential for autophagosome formation (Funderburk et al., 2010; He and Levine, 2010), have been reported to be reduced, along with PI3K, in brains from individuals with AD, although not in brains from two different mouse models of AD in which the *App* gene was overexpressed (Pickford et al., 2008). The deletion of beclin-1 in an APP transgenic mouse model decreased neuronal autophagy, increased intraneuronal Aβ and induced neurodegeneration associated with significant accumulations of abnormal lysosomal compartments. By contrast, overexpressing beclin-1 in these mice decreased the extent of amyloid pathology. A reduction in beclin-1 levels in AD, if confirmed, would be expected to reduce autophagosome formation, although given the multiplicity of actions of Beclin-Vsp34 core complexes on vesicular trafficking, the expected effects of lowering beclin-1 on AD pathology in the mouse model are not straightforward. Indeed, beclin-1 deficiency in this study also leads to impaired AV clearance by lysosomes, which may explain why amyloid pathology was exacerbated even though autophagosome formation was apparently reduced.

In summary, upregulation of autophagy induction in AD is supported by some, but not all analyses of mTOR activation state and is consistent with the autophagy response that might be expected in the face of increased numbers of damaged proteins and organelles in aging and diseased neurons. An increased delivery of substrates into the autophagy pathway would be expected, in turn, to compound the problem presented by the impaired AV clearance seen in the AD brain.

Conclusion

The pathology of the autophagic-lysosomal system in the AD brain and in AD models is uniquely extensive among late age onset neurological disorders, strongly suggesting a profound disturbance of autophagy-related functions. Multiple lines of evidence support the conclusion that the efficiency of lysosomal/autolysosomal proteolysis of autophagy substrates is markedly impaired by possibly multiple factors, which include the direct disruption of lysosomal acidification/proteolysis by PS1 mutations that cause early onset AD. Although a defect in AV and autophagic substrate clearance is sufficient to account for the robust accumulation of AVs and incompletely digested proteins in AD, there is additional evidence that autophagy may be upregulated and that increased levels of substrates may reach autolysosomes due to a pathological acceleration of endocytosis driven by genetic and environmental risk factors for AD. These are conditions that could compound the primary defect in clearance. This is a rapidly emerging field and new findings will further illuminate the underlying mechanisms of autophagy failure and no doubt identify additional contributing factors. A more complete understanding of autophagy regulation in the coming years will yield innovative approaches to modulate autophagy and evaluate their promise as therapies for AD and possibly other neurodegenerative diseases.

Acknowledgments

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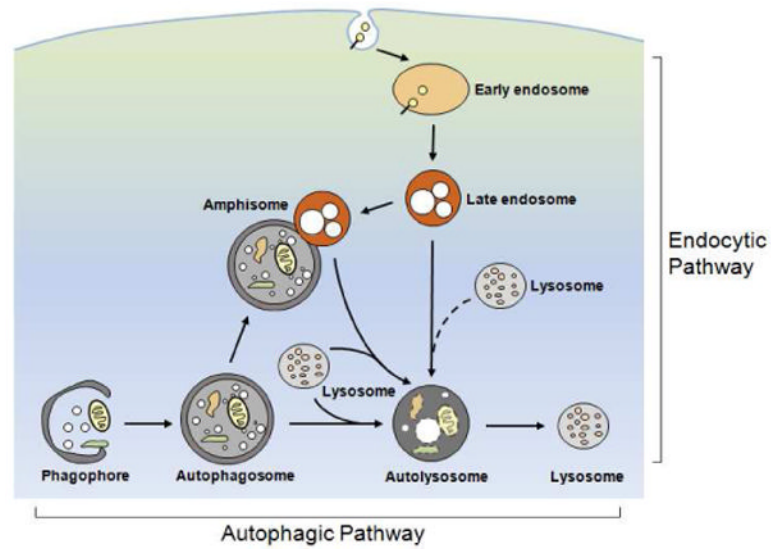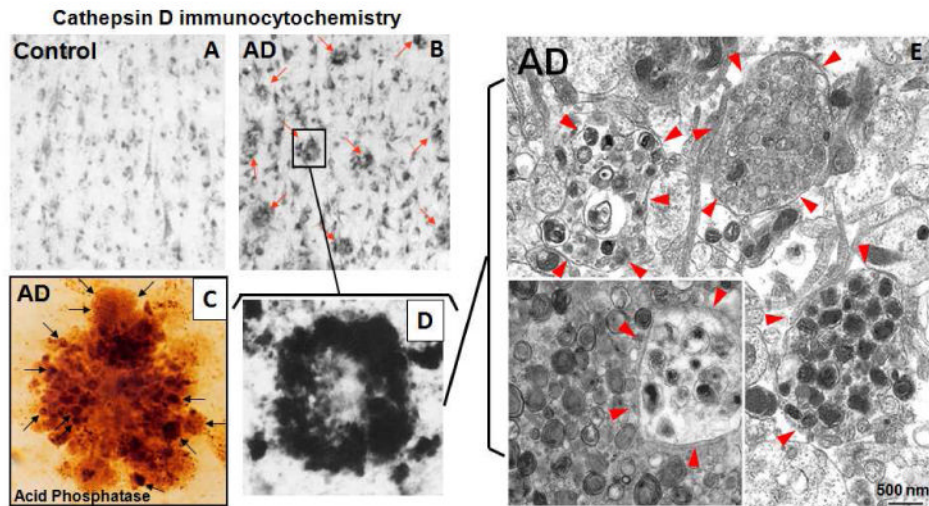
Figure 1.
Schematic illustrating the endocytic and the autophagic pathways to the lysosome.
Internalized materials entering the endocytic pathway are directed to early endosomes,
which mature to late endosomes/multivesicular bodies. In the autophagic pathway, a
phagophore sequesters an area of cytoplasm containing organelles to form a double
membrane-limited autophagosome. The formation of amphisomes via the fusion between
autophagosomes and late endosomes/multivesicular bodies provides an interactive point
between the two pathways. Autophagosomes, and amphisomes in the two pathways receive
hydrolases by fusing with lysosomes to form autolysosomes or late endosomes/
multivesicular bodies to form amphisomes. Efficient digestion of substrates within these
compartments yields lysosomes containing mainly acid hydrolases.
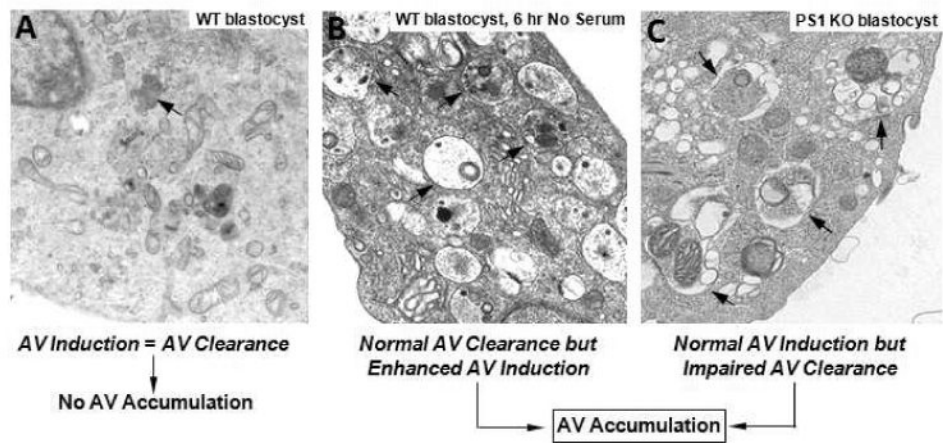
Figure 2.
Autophagy homeostasis requires a delicate balance between autophagosome formation and clearance. Despite a constitutive level of autophagic protein turnover, normal cells in their basal state display dense lysosomes (arrow) but relatively few autophagic vacuoles (A). Strong autophagy induction, in this case by acute serum deprivation, elicits the robust formation of autophagic vacuoles (AVs)(arrows) because autophagosome formation now exceeds the rate of AV clearance (B). The loss of the capacity to properly acidify lysosomes, as it exists in the blastocyst-lacking presenilin 1, markedly slows lysosomal proteolysis and AV clearance without altering autophagy induction (Lee et al., 2010), and AVs (arrows) robustly accumulate (C). Although both experimental conditions induce AV accumulation, albeit with somewhat different composition, the rate of autophagic protein turnover is much higher than normal in the serum-deprived cells and much lower than normal in the PS1 KO cells.

Figure 3.
The "burden" of AVs containing incompletely digested proteins stored in dystrophic neurites of the AD brain is great. At the light microscopic levels, CatD immunostaining in the AD brain (B) but not in the control brain (A) reveals a high density of senile plaques (B, arrows) each containing dozens of dystrophic neurites visible after histochemical staining for acid phosphatase (C, arrows) or by CatD immunocytochemistry (D). (E) Ultrastructural analysis of dystrophic neurites (circled by arrowheads) demonstrates that dystrophic neurites predominantly contain hundreds of AVs of distinct subtypes but the majority have double membranes with electron dense lumens, most consistent with autolysosomes and amphisomes in which proteolysis of substrates, including inner membrane, has stalled. Given the number of dystrophic neurites in the AD brain, the amount of "stored" wasted proteins is enormous, rivaling that in certain primary lysosomal storage disorders.