Modulation of Autophagy as a Therapeutic Target for Alzheimer's Disease

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Macroautophagy (autophagy) is a conserved cellular pathway that regulates the degradation of long-lived proteins, protein aggregates, and cellular organelles. Autophagy is essential for maintaining neuronal homeostasis; however, neuronal autophagic efficiency decreases with age. Therefore, aging is one of the greatest risk factors for development of Alzheimer's disease (AD), a slowly progressing form of neurodegeneration that develops over the course of 10-20 years prior to the onset of overt clinical symptoms. AD is defined neuropathologically by the presence of extracellular aggregates of the amyloidogenic protein amyloid-β (Aβ) and intracellular accumulation of the microtubule-associated protein tau. At end-stage Alzheimer's disease, abnormal autophagic pathology has been reported in human brain and in multiple mouse models of AD, suggesting that an intimate association may exist between neuronal autophagy stasis and Alzheimer's-related pathology. Here, we highlight recent evidence that the autophagic pathway plays a role in both the generation and clearance of the pathogenic Aβ protein and its precursors. The primary focus of this review is to examine the compelling research that highlights the autophagic pathway as a therapeutic target for AD and to discuss the therapeutic space around autophagy-regulating programs for AD. Finally, we propose that programs targeting autophagy regulation for AD ought to consider prophylactic or early stage intervention trials based on evidence against druggability of this pathway in late-stage disease.

Introduction

The macroautophagic/lysosomal pathway (referred to here as autophagy) is a conserved catabolic pathway, which regulates the degradation of long-lived proteins, aggregated proteins, and cellular organelles. In mature neurons, this catabolic pathway is regarded as constitutively active and normally efficient (Nixon and Yang, 2011). However, even minor perturbations along this pathway have been shown to stall autophagic activity and may impart a detrimental effect on cellular homeostasis of neurons (Yue et al., 2009). Autophagy is proposed to play a role in the pathogenesis of multiple neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases. Since most aggregate prone proteins (i.e. amyloid-β or α-synuclein) are high molecular weight structures, they are typically too large to enter the relatively narrow pore of the ubiquitin-proteasome barrel for degradation and have therefore become substrates of the autophagic pathway (Yue et al., 2009, Klionsky et al., 2011, Koga et al., 2011, Sridhar et al., 2011).

An age-dependent decrease in basal neuronal autophagic turnover has been associated with neurodegeneration, and evidence suggests that this perturbation of autophagic stasis may result in hyperaccumulation of protein aggregates (Koga et al., 2011, Sridhar et al., 2011). Recently, many studies have sought to understand the role of neuronal autophagic stasis in the pathobiology of Alzheimer's disease (AD), and the question arises as to the causative or symptomatic role of autophagy in AD. From these studies, we have gained a wealth of insight into the design of small molecule regulators of autophagy, novel therapeutic targets, and basic understanding of the course of the disease. Here, we provide

a summary of these findings, taking into consideration the relationship between autophagic stasis and AD pathology, "druggability" of the pathway at different stages of the disease, and outlook toward therapeutic space around the autophagic pathway for AD.

The Autophagic Pathway

Since autophagy was first reported more than 40 years ago (Cheung and Ip, 2011; Deter and De Duve, 1967), research focused on this major protein degradation system has aimed to elucidate the molecular mechanisms that are crucial for maintaining cellular homeostasis. Due to its involvement in both constitutive and stress-induced turnover of proteins and other cytoplasmic content, it is no surprise that studies implicate autophagy in the clearance of protein aggregates, the hallmark of many neurodegenerative diseases. More significantly, understanding the mechanisms and molecular controls for autophagy could provide useful targets in the treatment of such diseases.

Autophagy was first discovered through electron microscopy (EM) studies and, due to a lack of specific protein markers, for a long time remained as the only reliable method detect autophagic degradation in cells and tissue. Following the first published EM pictures showing dense mitochondrial structure in mouse kidney (Clark, 1957), a significant advance was made in the 1960s that revealed autophagic functions as a part of the lysosomal system and their contribution to numerous developmental and pathological conditions (Arstila and Trump, 1968, 1969). Even though technology has advanced to detect autophagy with various other techniques, EM retained its fundamental position in the detection of autophagic processes. For example, by using EM, Ericsson observed that ferritin is transferred to lysosomes following their sequestration by autophagic vesicles, proving that autophagic vesicles are nondegradative until they fuse with lysosomes (Ericsson, 1969). He also suggested that autophagy has a preeminent role under normal conditions, as well as under starvation in various organs including kidney, liver and brain.

Since these pioneering studies, much progress has been made in understanding the specifics of the autophagic pathways. Autophagy begins with the formation of the autophagosome, a double-membraned vesicle that sequesters the cytoplasmic content to be degraded. Such a vesicle is created from the extension of isolation membrane, otherwise known as a phagophore (Mizushima et al., 2002). While it remains unclear exactly where the isolation membrane comes from, it is generally believed to involve the endoplasmic reticulum, mitochondria, golgi apparatus and plasma membrane (Hayashi-Nishino et al., 2009; Hailey et al., 2010; Ravikumar et al., 2010; van der Vaart and Reggiori, 2010; Nixon and Yang, 2011). The autophagosome then fuses with either an endosome to form an amphisome, or with a lysosome to form an autolysosome, both of which degrade the sequestered material via acidic lysosomal hydrolases (Klionsky, 2007; Mizushima et al., 2008).

The process of autophagy is initiated by the assembly of various proteins, including Vps34, Beclin1, UVRAG, Vps15, Atg14, Ambra1 and Bif-1/endophilin B1 (Cheung et al., 2011), into a macromolecular complex in an intricate and precise manner. The processes of vesicle elongation and autophagosome formation that follow are also directed by a host of Atg proteins working in synchrony (Cheung et al., 2011). Even proteins previously not thought to be involved in autophagy have been found to have regulatory roles, including the small GTPase Rab5 (Ravikumar et al., 2008) and ubiquitin (Rothenberg et al., 2010). Autophagic control is further complicated by selectivity for certain cargoes through the use of association proteins (Tung et al., 2012). While the exact mechanisms are unclear, certain cargo receptors have been identified, including p62 and NRB1 (Pankiv et al., 2007; Knaevelsrud and Simonsen, 2010), that associate with autophagosomal marker LC3, effectively entrapping certain molecules for degradation (Pankiv et al., 2007; Ichimura et al., 2008; Kirkin et al., 2009; Tung et al., 2010).

Autophagic Pathology in Alzheimer's Disease

A plethora of studies confirm that autophagy is constitutively regulated in neurons, suggesting that any perturbation of this pathway might alter cellular homeostasis. The role of autophagy in neurons and its potential contribution to neurodegenerative disease pathologies have been extensively investigated by many groups (Nixon et al., 2000, Larsen and Sulzer, 2002, Boland et al., 2008, Jaeger and Wyss-Coray, 2009, Garcia-Arencibia et al., 2010). Among these, an AD-autophagy connection was made by several studies reporting the importance of the endo-lysosomal pathway in APP processing (Koo and Squazzo, 1994, Cataldo et al., 1997, Grbovic et al., 2003, Pasternak et al., 2003). However, the Nixon group was the first to show a link between autophagy and AD pathology using EM methodology (Cataldo et al., 1994), and they extended these findings by providing evidence for accumulation of immature autophagic vesicles in dystrophic neurites in the AD brain (Nixon et al., 2005, Nixon, 2007). In these pathologic brains, not only are there abnormally large pools of autophagic vacuoles (Nixon et al., 2005), but also pathogenic levels of Aβ accumulation within those vacuoles (Yu et al., 2005), suggesting that autophagy may be involved in the progression of AD. Indeed, a decline in multiple pathways of autophagy activity correlates tightly with normal aging, which has the similar phenotype of unwanted Aβ accumulation (Lipinski et al., 2010).

Generation and Degradation of Aβ via Autophagy

The amyloid precursor protein (APP) and its metabolites (APP-CTFs and Aβ) have been shown to colocalize with LC3 positive autophagosomes in APP-overexpressing cell lines (Lunemann et al., 2007). Evidence suggests that APP and its metabolites (notably the Aβ precursor βCTF and Aβ) are substrates of autophagy and are therefore subject to autophagy-mediated clearance (Figure 1; Tung et al., 2012). The levels of APP and its metabolites are decreased through autophagy activation by overexpressing Beclin1, a protein involved in autophagy initiation and autophagosome clearance (Jaeger et al., 2010; Tian et al., 2011). The same group also found that cells depleted of Beclin1 exhibit significant accumulation of Aβ and APP (Jaeger et al., 2010). Other groups report the accumulation of APP metabolites in the absence of the ATG5 and ATG7 genes (Tian et al., 2011, Steele et al., 2012b). These findings support the role of autophagy in the regulation of APP processing, suggesting that inhibition or ablation of autophagy may result in the detrimental accumulation of toxic substrates.

Intriguingly, other studies have found a therapeutic effect for ablation of autophagy in AD. Some evidence suggests that activation of autophagy leads to increased formation of Aβ within autophagic vacuoles, potentially exacerbating AD pathology, based on evidence that Aβ is generated from its precursor βCTF within autophagosomes (Yu et al., 2005). Additionally, autophagy has also been linked to increases in Aβ production during hypoxia (Li et al., 2009). Presenilin-1 and Nicastrin, key components of γ-secretase enzyme complex, were also shown to co-localize with APP and retain γ-secretase activity in lysosomal vesicles proving involvement of the endo-lysosomal pathway in APP processing (Pasternak et al., 2003). These data suggest that aberrant induction of autophagy may lead to accumulation of autophagic vesicles containing active γ-secretase machinery, thus elevating Aβ production (Yu et al., 2005, Boland et al., 2008). Specifically, PS1 mutations associated with familial early-onset AD are known to cause increased Aβ production, and this may lead to abnormal regulation of the autophagic pathway at multiple points, as discussed below. Consistent with this data, inhibition of autophagy has been shown to alleviate Aβ-induced cellular death (Hung et al., 2009).

Aβ as a Regulator of Autophagic Stasis

Some studies have focused efforts on understanding the role of pathogenic Aβ accumulation in the regulation of autophagic stasis. Studies implicate Aβ in the modulation

of autophagy, suggesting that, through either an Akt-dependent pathway or generation of reactive-oxidative species (ROS; Hayashi et al., 2009; Lipinski et al., 2010), Aβ may contribute to a feedback loop in maintaining its own homeostasis, triggering self-degradation when there is enough buildup in the autophagic vacuoles (Hung et al., 2009). However, in the presence of excessive amounts of Aβ, or higher ordered structures that are resistant to degradation (Knauer et al., 1992), such an accumulation can lead to lysosomal membrane permeabilization, resulting in ROS-induced apoptosis (Zheng et al., 2006a; Zheng et al., 2006b; Zheng et al., 2009). Recent evidence suggests that accumulation of insoluble Aβ in mouse brain may be sufficient to impair autophagic clearance, leading to further hyperaccumulation of Aβ and other autophagic substrates including α-synuclein (Lai and McLaurin, 2012, Steele et al., 2012a, Steele et al., 2012b). Together, these data suggest that the involvement of autophagy in AD is a complicated and multifaceted affair and that the autophagic pathway may be responsible for both the generation and degradation of Aβ, causing difficulty in determination of directional therapeutic

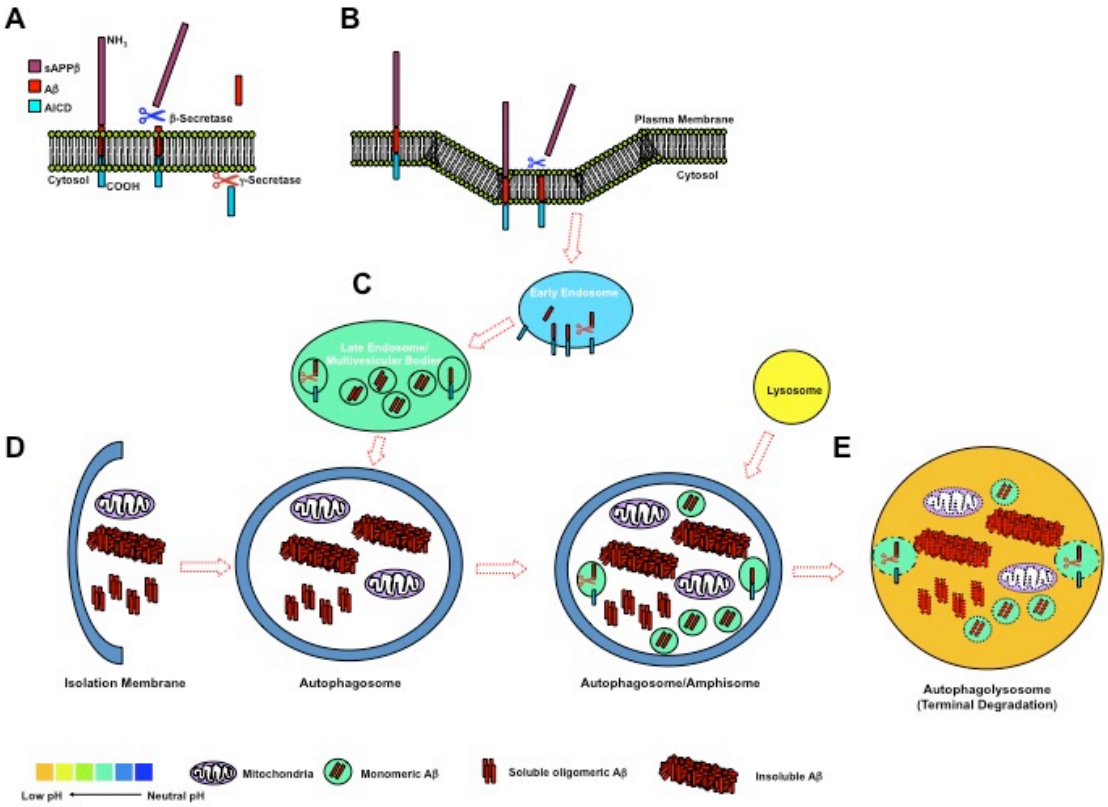

Figure 1. Therapeutic intervention strategies along the amyloidogenic pathway for metabolism of APP. (A) Along the amyloidogenic pathway, APP is processed first by the β-secretase (BACE-1) to generate a soluble N-terminal ectodomain and a membrane-bound C-terminal fragment (βCTF). The βCTF is then cleaved by the γ-sectretase complex to generate the Aβ peptide and the APP intracellular domain (AICD). (B) Cleavage of full length APP by BACE-1 typically occurs in the slightly acidic environment of the early endosome, and subsequent γ-secretase cleavage of the βCTF occurs within a variety of subcellular compartments thereafter. (C-E) Evidence suggests that Aβ may be both generated and degraded within the autophagic pathway. Multiple targets of intervention include inhibition of BACE-1 or γ-secretase actvity (A), disruption of endosomal trafficking (B,C), enhancement of autophagic turnover (D), or enhanced lysosomal protease activity (E). The latter two are the subject of this review.

targeting (Figure 1).

Autophagy Induction as an Essential Regulator of AD Pathology

Studies of mutant mice lacking autophagy-essential genes provide insight into the crucial role of autophagy in maintaining neuronal homeostasis. While the conventional knock out of autophagy-essential genes such as ATG5 or ATG7 lacks viability (Kuma et al., 2004, Komatsu et al., 2005), two critical studies have reported deletion of these genes conditionally in neurons (Hara et al., 2006, Komatsu et al., 2006). Studies of these conditional knockout lines showed similar pathological changes, growth retardation, motor and behavioral abnormalities and accumulation of ubiquitinated inclusions due to a lack of autophagy induction. Together with the observation that proteosomal function was not affected, abundance of ubiquitinated protein aggregates indicated an important role of autophagy in cytosolic protein turnover (Hara et al., 2006, Komatsu et al., 2006).

Autophagic contribution to turnover of ubiquitinated protein aggregates has also been addressed through the link between the role of p62 in ubiquitinated protein aggregate formation (Gal et al., 2007, Komatsu et al., 2007) and autophagic clearance of p62-labeled protein aggregates (Seibenhener et al., 2004, Bjorkoy et al., 2005). p62 is an autophagic cargo receptor, which was shown to bind directly to LC3 (Pankiv et al., 2007). In a recent study, Du et al. observed a significant decrease in p62 expression in a triple transgenic mouse model of AD (3xTg-AD; (Du et al., 2009). In addition, a genetic study with p62 knockout mice reported enhanced tau hyperphosphorylation in hippocampus and neocortex of 6-month-old mice (Ramesh Babu et al., 2008). Hyperaccumulation of p62 was reported in the absence of Atg5 or Atg7, and is associated with the accumulation of other typical autophagic substrates such as APP metabolites and α-synuclein (Steele et al., 2012a, Steele et al., 2012b). High levels of insoluble p62 were noted along with hyperaccumulation of LC3-II, Aβ and α-synuclein only after the onset of plaque deposition and cognitive defect (Steele et al., 2012b) in brain 4 month old TgCRND8 mice, which has been independently validated through EM studies of TgCRND8 mice at 4 months of age (Lai and McLaurin, 2012). Taken together, it appears that p62 may represent one biomarker for autophagic stasis and future studies ought to investigate whether soluble cerebrospinal fluid levels of p62 correlate with AD pathology and cognitive defect.

Another component of the autophagy pathway, Beclin 1 (also known as Atg6), has been studied to highlight the possibility that reduction in autophagy may be causative of AD.pathology. Beclin-1 is a proautophagic protein that is a component of a PI3 kinase complex and plays a vital role in autophagosome formation (Funderburk et al., 2010, He and Levine, 2010). While reduced beclin-1 level was reported in AD patient brains, deletion of beclin-1 in an APP transgenic mouse model of AD resulted in elevated intraneuronal Aβ accumulation, decreased neuronal autophagy, accumulation of abnormal lysosomal structures, and neurodegerneration (Pickford et al., 2008). Conversely, overexpression of beclin-1 reduced Aβ pathology (Pickford et al., 2008). Overall, these findings suggest that reduced induction of autophagy may contribute to Aβ pathology through the ablation of an essential pathway for degradation of cytotoxic protein aggregates.

Targeting the mTOR Pathway to Induce Autophagy in Alzheimer's Models

Autophagy is a highly regulated cellular maintenance pathway that is responsive to nutrient deprivation, as well as growth factor signaling pathways and other cellular homeostasis pathways. Amongst these, the mTOR (mammalian target of rapamycin) pathway plays a well-established, key regulatory role in nutrient sensing and cell metabolism, integrating many signaling cascades in the cell (Noda and Ohsumi, 1998, Corradetti and Guan, 2006). mTOR is a kinase

component of two complexes called mTOR complex (mTORC) 1 and 2 (Loewith et al., 2002). Rapamycin is an inhibitor of mTORC1 that controls cellular homeostasis, whereas mTORC2 is a modulator of actin dynamics and is not inhibited by rapamycin (Noda and Ohsumi, 1998, Loewith et al., 2002). The mTOR pathway has been shown to regulate both synthesis and degradation of proteins; thus it has a crucial role in neuronal pathways and is known to be involved in learning and memory (Tischmeyer et al., 2003, Ehninger et al., 2008). Positive regulators of mTOR, such as insulin and growth factors, signal through AKT and have an inhibitory effect on autophagy (Corradetti and Guan, 2006, Young et al., 2009). Upon its activation, mTOR induces protein synthesis and inhibits autophagy via Atg13 phosphorylation (Pattingre et al., 2008). On the contrary, energy depletion and increased calcium are negative regulators of mTOR through AMP-activated protein kinase (AMPK) that keep mTOR inactive, thereby activating autophagy (Inoki et al., 2003, Hoyer-Hansen et al., 2007, Pattingre et al., 2008).

In a recent study, Bharadwaj et al. (2012), using an *in vitro* GFP-Aβ42 yeast expression system, showed that treatment with either rapamycin or latrepirdine corrected Aβ42-induced cytotoxicity via enhanced autophagy. This study further reported that rapamycin or latrepirdine were capable of reducing levels of intracellular Aβ42 aggregates in wild-type yeast stains, but not in Atg8Δ cells. Another recent study indicated the efficacy of rapamycin or latrepirdine treatment in the protection of yeast cells from α-synuclein-induced toxicity. Multiple studies have been published to indicate that rapamycin (discussed below) or latrepirdine (Bharadwaj et al., 2012, Steele et al., 2012a, Steele et al., 2012b) stimulate mTOR- and Atg5-dependent autophagy, resulting in reduced levels of aggregated proteins and correction of cognitive defect in AD transgenic mouse models.

Caccamo et al. recently reported that mTOR activity is elevated in the neocortex and hippocampus of 3xTg-AD mice. Rapamycin treatment rescues the cognitive deficits in these mice and improves Aβ and tau pathology by restoring mTOR signaling (Caccamo et al., 2010). Similarly, Spilman et *al.* observed reduced Aβ levels and ablation of AD-like cognitive deficits in the PDAPP transgenic mouse model due to long-term inhibiton of mTOR by rapamycin (Spilman et al., 2010). A more recent study futher investigated the effects of autophagy induction on AD pathology in 3xTg-AD by comparing the rapamycin effect in 2-month-old and 15-month-old mice (Majumder et al., 2011), suggesting that rapamycin, when administrated as a preventative agent, induces autophagy and thus abrogates development of plaques, tangles and cognitive deficits.

Amongst Tau models of AD-like and frontotemporal dementia-like (FTD) neuropathology, recent evidence suggests that methylthioninium chloride (methylene blue) (Congdon et al., 2012) or trehalose (Kruger et al., 2012) may induce autophagy and attenuate tauopathy both in vitro and in vivo. Whereas methylene blue is suggested to activate autophagy through an mTOR-dependent mechanism, trehalose is proposed to act via an mTOR-independent mechanism. Both compounds have been shown to induce autophagy, albiet through different mechanisms, leading to the enhanced clearance of aggregated Tau and reduction of other autophagic substrates including p62. Further investigations of the autophagy-enhancing properties of methylene blue and trehalose may derive potent pro-autophagic molecules with utility in multiple neurodegenerative diseases, including AD and FTD.

Impaired Lysosomal Clearance Contributes to AD Pathology

Aberrant clearance of lysosomal substrates, including autophagosomes, has been heavily cited as a defect associated with the progression of AD (Knauer et al., 1992, Yang et al., 1995, Gouras et al., 2000, Yu et al., 2004, Yu et al., 2005, Boland and Nixon, 2006, Lee et al., 2010, Ma et al., 2010, Caccamo et

al., 2011, Majumder et al., 2011, Yang et al., 2011a, Yang et al., 2011b). Indeed, the near complete replacement of normal neuronal cytoplasmic contents by uncleared autophagosomes in dystrophic neurites and an overall increase in autophagosome accumulation in less affected neurites have been noted (Nixon and Yang, 2011). Generally, the field is at odds over which stage (if specifically attributable to any *one* stage) of the autophagic-lysosomal pathway is dysfunctional in AD.

Compelling evidence has emerged to support the claim that autophagy dysfunction in AD occurs due to defective lysosomal clearance (Yang et al., 2011a, Yang et al., 2011b). Substantial evidence for the aberrant accumulation of APP metabolites following lysosomal dysfunction comes from a series of studies in which the accumulation of autophagosomes and neuritic dystrophy is observed when lysosomal degradation is inhibited by deleting one or more cathepsins, by using cysteine protease inhibitors, or general lysosomal inhibitors (Nixon and Yang, 2011). Nixon and colleagues (Yang et al., 2011a) have extended these studies in the TgCRND8 mouse to indicate that promotion of lysosomal stasis ablates the accumulation of autophagic substrate. Moreover, the study of primary lysosomal storage disorders (GM1, GM2, Niemann Pick type C disease, and neuronal ceroid-lipofuscinosis) provides a link between similarities in cellular AD pathologies and these disorders, including elevated levels of APP metabolites (Nixon and Yang, 2011).

Another body of work indicates that genetic inhibition of autophagy upstream of lysosomal fusion (i.e. deletion of *ATG5* or *ATG7*) also results in the cellular accumulation of APP metabolites, suggesting that perturbation at any point along the autophagic and/or lysosomal pathway may result in AD-like pathology (Tian et al., 2011). Interestingly, the bulk of these studies have also implicated autophagic/lysosomal dysfunction in the pathogenesis of Parkinson's and Huntington's diseases, which involve the abnormal accumulation of α-synuclein and huntingtin, respectively (Cuervo et al., 2005, Boland and Nixon, 2006, Sarkar et al., 2007, Mak et al., 2010, Nixon and Yang, 2011). Recent work indicates that PS1 may play a critical role in lysosome acidification because conditional PS1 deletion resulted in elevated levels of autophagy substrates, inhibited autophagic turnover, and reduced cathepsin-specific activity (Esselens et al., 2004, Wilson et al., 2004). Fibroblasts derived from patients with *PSEN1* mutations also exhibited defective lysosome acidification, similar to that seen in *PSEN1* -/- cells (Lee et al., 2010). As discussed above, it has also been established that autophagic clearance of both APP metabolites and α-synuclein can be modulated via Atg5-dependent autophagy (Sarkar et al., 2007, Tian et al., 2011).

Indeed there is support of a critical role of lysosomal failure in the progression of AD, where severe autophagy neuropathology develops in all forms of AD, and also in mouse models of AD harboring only FAD-related APP mutations (reviewed in depth in (Nixon and Yang, 2011). Based on this line of evidence, it is possible that, in some instances, autophagic dysfunction develops over time and may be secondary to the accumulation of insoluble Aβ42 (Steele et al., 2012b). In support of this hypothesis, a recent report provides evidence that when TgCRND8 mice were treated prior to onset of autophagic/lysosomal failure with *scyllo*-inositol (SI), an endogenous inositol steroisomer that is known to inhibit aggregation and fibril formation of Aβ42, no converstion to autophagic/lysosomal failure was observed, by comparison to vehicle-treated TgCRND8 littermates, which significanlty accumulated uncleared autophagic vacuoles and autophagic/lysosomal substrates (Lai and McLaurin, 2012).

Recently, the Oddo laboratory published evidence indicating that the 3xTg-AD mouse model develops autophagic neuropathology by 15 months of age (Majumder et al., 2011). Briefly, 3xTg-AD mice received chronic rapamycin therapy beginning at either 2 months of age (pre-autophagic pathology) or 15 months of age (with autophagic pathology) and until the mice were 18-months-old

(Majumder et al., 2011). Chronic rapamycin therapy of 2-month-old 3xTg-AD mice enhanced memory, stimulated autophagy, and mice receiving rapamycin developed significantly fewer Aβ plaques and neurofibrillary tangles, whereas older (15-months-old) mice received no benefit or rapamycin therapy (Majumder et al., 2011). Based on this finding, we suggest that stimulation of mTOR-dependent autophagy prior to the onset of autophagic dysfunction would represent a viable prophylactic strategy, but a stategy which is unlikely to lead to success as a late-stage intervention (Majumder et al., 2011, Yang et al., 2011a, Yang et al., 2011b, Steele et al., 2012b).

Development of Small Molecule Regulators of Autophagy

Protein aggregates are highly ordered, resistant to degradation, and substrates of autophagy (Glabe, 2008). Therefore, enhancement of autophagy as a theapeutic avenue in the treatment of neurodegenerative diseases has led to the discovery of small molecule regulators of autophagy, including several previously FDA-approved therapeutics (Zhang et al., 2007). One such group of autophagy-inducing molecules are the small-molecule enhancers (SMERs) of rapamycin (Sarkar et al., 2007). Through a large screen of compounds in yeast, three SMERs were found to increase autophagy, which directly correlated with a decrease in mutant huntintin aggregates in *Drosophila* models of Huntington Disease (Sarkar et al., 2007). Their counterparts, small-molecule inhibitors of rapamycin (SMIRs), were found to decrease the clearance of the autophagic substrate α-synuclein (Sarkar et al., 2007). Recently, SMER28, a bromo-substituted quinazoline, was found to promote the degradation of Aβ and APP-CTFs through increased autophagic clearance utilizing essential autophagic components such as Atg5, Beclin1, and Ulk1 (Tian et al., 2011). Several SMERs, including SMER10, SMER18, and SMER28 were also shown to enhance the degradation of α-synuclein via Atg5-dependent autophagy, suggesting that autophagy might represent a common mechanism for clearance of both APP metabolites and α-synuclein (Sarkar et al., 2007). The discovery and role of autophagy modulators for aggregate clearance in neurodegenerative diseases is a viable therapeutic strategy, which will continue to move forward in generating novel compounds for autophagy regulation.

Conclusions

The study of autophagy in neurodegenerative diseases has increased dramatically over the past decade. The autophagic pathway is a conserved and essential mechanism for maintenance of cellular homeostasis and the clearance of long-lived proteins, protein aggregates, and dysfunctional cellular organelles – all of which contribute to the pathobiology of Alzheimer's disease. Here, we discussed the many recent findings, which link alteration of autophagic stasis with Alzheimer's pathology in cultured cell models, mouse models, and in AD patients. However, many questions remain to be answered regarding the role and, importantly, the therapeutic value of autophagy in AD. For example, failure is evident in the brains of patients with late-stage AD to the point that accumulated autophagosomes occupy the bulk of neuronal cytosol (Yu et al., 2004, Boland and Nixon, 2006, Nixon and Yang, 2011), however it is unclear whether autophagic failure is causative of canonical AD pathology early on in the disease or whether AD pathology leads to the failure of the autophagic pathway.

Several studies have indepedently addressed this key issue. Multiple groups have shown that deletion of autophagy-essential genes ATG5 or ATG7, or alteration in levels of beclin-1 results in accumulation of APP metabolites (including Aβ), suggesting that Aβ is generated within the autophagic pathway and subsequently delivered to the lysosome for degradation. Research from the Nixon group and others suggests that AD-related PS1 mutations result in defective lysosomal clearance, resulting in accumulation of Aβ (Yang et al., 2011a, Yang et al., 2011b). Studies of lysosomal storage diseases indicate

that lysosomal failure is sufficient to cause accumulation of Aβ (Keilani et al., 2012) similarly to PS1 mutations or pharmacological inhibition of lysosomal acidicifation. These studies also indicate the high levels of γ-secretase localization and activity in lysosomes, suggesting the autophagic pathway and lysosome as a likely locus for Aβ generation and accumulation. Taking all of these studies into consideration, we suggest that the entire autophagic pathway from induction to maturation and clearance is essential for regulating the normal turnover of APP and its metabolites, and, importantly, that perturbation of this pathway, whether pharmacological, mechanical, or genetic, results in hyperaccumulation of autophagic substrates.

The field seems to have reached a chicken-or-egg scenario in regards to the role of autophagic failure in the pathogenesis of AD (Figure 1). Recent evidence indicates that onset of cognitive failure, and the deposition of insoluble Aβ, precedes the apparent failure of the autophagic pathway in at least one mouse model of AD (Steele et al., 2012b). This study suggests that insoluble Aβ aggregates may be resistant to autophagic degradation and toxic to this essential pathway, leading to its failure and subsequent cytotoxic hyperaccumulation of substrate. Support is lent to this argument by other studies which indicate the loss of efficacy of rapamycin therapy only after the onset of insoluble plaque deposition in another AD mouse model (Caccamo et al., 2011, Majumder et al., 2011). Regardless of the cause, it is plausible that autophagic failure, which results in hyperaccumulation of autophagic substrates, may cause the acceleration of clinical symptoms at mid-to-late stage disease due to increased intraneuronal pathology.

Alzheimer's disease is a slowly progressing neurodegenerative disease, which develops over the course of 10-20 years prior to onset of overt clinical symptoms (Gandy, 2012). In consideration of therapeutic strategy to target cellular maintenance pathways (e.g. autophagy), one must consider the functionality of these pathways in respect to stage of neuropathology. Autophagy enhancing therapies such as rapamycin, SMER-28, and latrepirdine promote degradation of Aβ in cultured cells and have been shown to reduce cytotoxicity in vitro. In vivo studies with latrepirdine or rapamycin suggest that there is a druggable window during which a pro-autophagic strategy is efficacious, and that this efficacy is lost at late stage disease due to failure of autophagic clearance. Based on these studies, it appears evident that design of pro-autophagy therapeutics ought to focus on prophylaxis or early-stage intervention for AD, bearing in mind that these drugs may add little therapeutic value as late-stage interventions.

Finally, it should be noted that the therapeutic space around autophagy-regulating therapeutics for neurodegenerative disease remains vast. To date, very few scaffolds have been identified which regulate autophagy, and few of these have been shown to penetrate the blood-brain-barrier efficiently. Whereas the focus of this review regards the targeting of autophagy as a potential AD therapeutic strategy, autophagy-regulating therapeutics have been proposed for use in combatting many other CNS and peripheral diseases, including cancers and fibrogenesis (Hernndez-Gea and Friedman, 2012, Hilscher et al., 2012). Development of efficient, blood-brain-permeable small molecule regulators of autophagy will dramatically influence the landscape of prophylactic Alzheimer's therapies, although many challenges stand in the way. Standardization of techniques for measuring autophagic activity has begun (Klionsky et al., 2012) and new studies continue to elucidate important therapeutic targets, molecular mechanisms, and implications for when and how to target autophagy in many diseases.

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