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Autophagy-Derived Alzheimer's Pathogenesis

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1. Introduction

Alzheimer's disease (AD) is an incurable terminal neurodegenerative disorder primarily affecting the elderly. Even after a century of intensive investigation, its pathogenic mechanism still remains enigmatic. Many hypotheses have been advanced to interpret the disease pathogenesis; however none are able to provide an integrated mechanistic view that can unify the numerous superficially disconnected aspects of AD etiology and pathology. Extracellular amyloid plaques and intracellular neurofibrillary tangles are the two prominent hallmarks of AD neuropathology. It remains unclear what pathogenic events link aggregated proteins such as amyloid beta peptides (Aβ) and/or phosphorylated tau to neuronal damage and death. It is also important to know more precisely how advancing age triggers the disease pathogenesis and how other modifiers affect the disease process. The absence of this basic knowledge is a major barrier not only for understanding of the disease but also for development of effective AD therapies.

Autophagy, or specifically macroautophagy, is a subcellular process participating in membrane trafficking and intracellular degradation and functions in the turnover of damaged organelles and unfavorable proteins through the lysosomal machinery. The autophagy-lysosomal system plays an important role in maintaining intracellular homeostasis and also participates in the pathophysiology of many diseases including cancer, infectious and neurodegenerative diseases (Mizushima et al., 2008). Abnormal autophagic structures have been reported to be extensively involved in AD pathology in brains of human patients as well as animal models (Nixon et al., 2005; Shacka et al., 2008). However, it remains unclear how autophagy contributes to the disease.

Numerous review papers are available that summarize the current knowledge regarding the molecular and cellular aspects of autophagy and its extensive involvement in various diseases. In this chapter, we focus on the concept of an "autophagy-lysosomal cascade" as a key mechanistic insight into AD pathogenesis. This disease hypothesis is based on recent work from our laboratory as well as growing evidence from other AD research groups. The autophagy-lysosomal cascade hypothesis has the capability to integrate many seemingly disconnected aspects of AD pathophysiology into a common cellular framework. We believe that further characterization of the details of autophagic participation in AD will be important for development of anti-Alzheimer's therapies.

2. Autophagy-derived Alzheimer's pathogenesis: Signs, lesions and causes

Autophagy-lysosomal involvement in AD and other related animal models has been extensively documented. However, it remains enigmatic if autophagy plays a causative role

or is a consequence of the disease process. It is also unclear if autophagy is protective or detrimental with respect to the disease pathogenesis. AD has a multifactorial etiology and also exhibits heterogeneous pathological signs. Correspondingly, numerous disease hypotheses have been proposed primarily based on one or few particular pathological features; currently, no hypothesis can provide a unified mechanistic connection to the hierarchical changes in AD pathogenesis. Practically, an accurate disease mechanism is expected to be attributable to different aspects of the disease etiology and also interpretable to the development of different pathological features of the disease. Here we introduce an autophagy-lysosomal cascade in AD pathogenesis and discuss how this pathogenic cascade is initiated by or contributes to the different aspects of the causes, the signs and the lesions of AD pathophysiology.

2.1 Granulovacuolar degeneration and autophagy-lysosomal neuropathology

Granulovacuolar degeneration (GVD) along with plaques and tangles are the earliest described and also the most prominent histopathologic signs of AD (Anderton, 1997; Ball, 1982; Burger & Vogel, 1973; Funk et al., 2011; Okamoto et al., 1991). Granulovacuolar structures were initially reported for AD in 1911. They are characterized as large translucent vacuoles containing electron-dense granule cores appearing in cytoplasm (Shacka et al., 2008) and are often found in pyramidal neurons of the hippocampus. GVD bodies are double membrane enclosed partially digested cytoplasmic contents (Okamoto et al., 1991), suggesting an autophagic origin for the GVD. This autophagic association is further confirmed by positive immunostaining for LC3 and p62 (autophagic markers), LAMP1 (lysosome-associated membrane protein 1) and CHMP2B (charged multivesicular body protein 2B) to the GVD bodies (Funk et al., 2011; Yamazaki et al., 2010). These studies suggest that the GVD bodies are enlarged vesicles derived from autophagy and endocytosis. GVD may also appear in the normal aging brains where plaques and tangles are sparse (Anderton, 1997).

One of the earliest pathological signs observed in patients with AD is the appearance of numerous enlarged autophagic and endosomal vesicles accumulating in perikarya, neurites and synaptic terminals (Nixon et al., 2005; Nixon et al., 2008; Shacka et al., 2008) due to defective autophagy-lysosomal degradation. The defect was initially thought to result from a putative blockage of vesicle fusion among autophagosomes, endosomes and lysosomes thus leading to the failure for autophagosomes to acquire lysosomal catabolic enzymes necessary for cargo digestion (Boland et al., 2008; Nixon, 2007; Nixon et al., 2005; Yu et al., 2005). This view was primarily based on distinguishing autophagosomes in electron micrographs. The identification of pre- and post-lysosomal autophagic or endosomal vesicles in electron micrographs may be misleading. Distinct types of vesicles can dynamically fuse with each other and thus form diverse highly polymorphic structures. These heterogeneous vesicles are hard to be identified with certainty, especially when compromised as part of the disease process. Failure of lysosomal acidification was also proposed as an alternative mechanism responsible for defective autophagic degradation (Lee et al., 2010). By direct expression of human $A\beta_{1-42}$ in *Drosophila* brains, we found that some dysfunctional autophagic vesicles have clearly fused with lysosomes and are acidified (Ling et al., 2009). Thus the massive accumulation of autophagy-lysosomal vesicles in brains apparently results from the vesicular storage of indigestible cargo including $A\beta_{1-42}$ aggregates and other contents. Lysosomal-derived secondary lesions caused by the vesicular leakage of the autophagy-lysosomal contents into cytosol may further activate autophagy and exacerbate vesicle accumulation as discussed in the next section.

Direct Aβ$_{1-42}$ expression in *Drosophila* brains induces age-dependent neurodegeneration through an autophagy-lysosomal injury (Ling et al., 2009). Many degenerative neurons exhibit typical granulovacuolar features (Fig. 1), reflecting the reliability of the *Drosophila* model as an important tool to dissect AD pathogenic mechanisms. In addition to the prominent GVD, the neuronal autophagy-lysosomal machinery may also contribute to the development of amyloid plaques (our unpublished observation) as well as other disease-associated phenotypes such as tangle formation, neurite atrophy, synapse loss, etc as discussed in the following sections. Taken together, autophagy-lysosomal involvement in AD is an early histopathologic sign that has been well recapitulated in different animal models of AD.
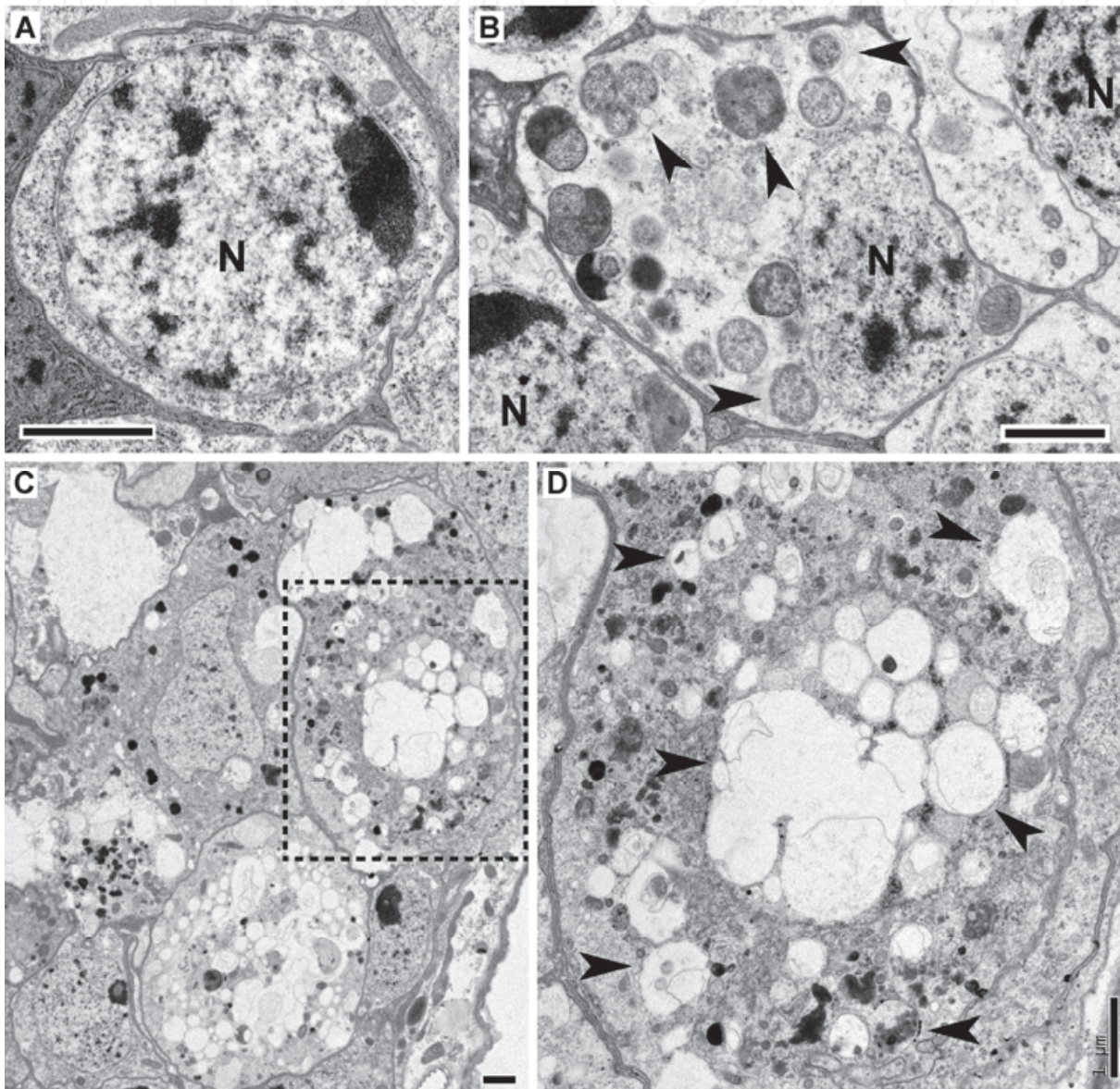

Fig. 1. The GVD morphology recapitulated in a *Drosophila* AD model with neuron-limited expression of human Aβ$_{1-42}$. (A) A normal morphology of neuronal soma. (B) An affected neuron accumulates numerous autophagy-lysosomal vesicles. (C) Extensive neurodegeneration occurs with GVD feature. (D) A higher magnification of view of the neuronal soma in the square area in (C). Arrowheads, autophagy-lysosomal vesicles; N, nuclei. Scale bars = 1μm.

2.2 The pathogenic lesions of AD are a result of the autophagy-lysosomal injury

AD exhibits heterogeneous features in its clinical symptoms, histopathology and neurochemistry. Besides the GVD discussed above, other well-documented neuropathological changes include widespread neuron loss, extracellular plaques, intraneuronal tangles, Hirano bodies, defective mitochondria, neurite atrophy, synapse loss, calcium dyshomeostasis, oxidative stress, neuroinflammation, cerebral amyloid angiopathy, etc. The cause-effect relationships between or among these changes have never been clearly established. To clarify the cause-effect relationships among these changes related to neuronal autophagy, we classify them here as pathological signs or pathogenic lesions. A pathological sign is defined as any detectible pathological event not resulting in additional downstream pathological events; whereas a pathogenic lesion is defined as any detectible pathological event causing other downstream pathological events. Previously we proposed a central role of autophagy-lysosomal system in AD pathogenesis (Ling & Salvaterra, 2009). Here we discuss how a primary autophagy-lysosomal injury in neurons might sit at the top of a pathogenic hierarchy and initiate the secondary and tertiary lesions such as mitochondrial dysfunction, oxidative stress, intracellular Ca^{2+} dyshomeostasis, membrane and organelle damage, all of which eventually develop into the plethora of heterogeneous neuropathologic signs including neurological defects, extracellular diffuse Aβ deposition, amyloid plaques, intracellular tangles, Hirano bodies, neurite and synapse atrophy, extensive neuronal death, etc.

2.2.1 Amyloid deposition and autophagy-lysosomal machinery

A widely held view is that Aβ is produced via APP proteolysis at the surface of neuronal cytoplasmic membranes and released into extracellular spaces. Diffusely distributed extracellular Aβ then assembles into toxic oligomers, aggregates and eventually condenses into senile plaques over a long period of time (Armstrong, 1998; Marchesi, 2005; Torp et al., 2000). However, emerging evidence has demonstrated that a large fraction of Aβ is generated in intracellular compartments rather than at cell surfaces (Gouras et al., 2005; LaFerla et al., 2007). Several subcellular loci have been suggested for intracellular Aβ production including rough endoplasmic reticulum (ER), Golgi apparatus, endosomes, autophagosomes and lysosomes. However, it is unclear how intracellular Aβ is subsequently transported to extracellular spaces and how Aβ deposits into the focal amyloid plaques (Fiala, 2007; Gouras et al., 2005).

The intracellular Aβ may be sequestered by autophagy-lysosomal machinery along with damaged organelles where Aβ is generated. We previously showed that autophagy-sequestered Aβ$_{1-42}$, in turn, decreases the capacity of autophagy-lysosomal degradation (Ling & Salvaterra, 2011a; Ling et al., 2009). Aβ$_{1-42}$-induced dysfunction of lysosomal vesicles may retain indigestible Aβ$_{1-42}$ along with Aβ$_{1-40}$. In support of this possibility, highly concentrated intracellular Aβ has been identified in various autophagic and endosomal vesicles (Petanceska et al., 2000; Takahashi et al., 2002). Autophagy-lysosomal compartments also function in secretion (Gerasimenko et al., 2001; Griffiths, 2002; Luzio et al., 2007; Manjithaya & Subramani, 2011; Pfeffer, 2010). It is plausible therefore that some of lysosomal vesicles may secret their stored monomeric or oligomeric Aβ peptides into extracellular spaces. Consistent with this, some early observations showed that Aβ is secreted by intracellular secretory compartments (Probst et al., 1991; Rajendran et al., 2006). Highly concentrated Aβ$_{1-42}$ aggregates stored in enlarged autophagy-lysosomal vesicles may

contribute to the development of amyloid plaques during aging or neurodegeneration (our unpublished observation). Thus the neuronal autophagy-lysosomal pathway appears to play a central role in amyloid deposition associated with either AD or normal brain aging.

2.2.2 Lysosome-derived chemical lesions and subcellular damage

Aβ (especially Aβ_{1-42}) is an amphipathic molecule known to disturb biological membranes (Eckert et al., 2010; Gibson Wood et al., 2003). The membranes of lysosome-related vesicles with an acidic microenvironment are especially sensitive to Aβ disturbance (Ditaranto et al., 2001; McLaurin & Chakrabartty, 1996). This membrane disruption is thought to result from the direct interaction between the hydrophobic C-terminus of Aβ peptides and the lipid bilayer of the membrane (Marchesi, 2005). The interaction also appears to be important for membrane-associated Aβ assembly into higher ordered structures (Friedman et al., 2009; Sureshbabu et al., 2010). Compromised membrane integrity greatly increases membrane conductance that has been attributed to a putative ionic channel formed by Aβ peptides (Jang et al., 2010).

Aβ_{1-42} expressed in *Drosophila* brains induces a deterioration and compromise of autophagy-lysosomal vesicles (Ling et al., 2009). The vesicle compromise and subsequent leakage is a primary causative event that results in secondary pathogenic lesions as evident by extensive membrane disruption occurring in cytoplasmic, nuclear and other organelle membranes. In electron micrographs, disrupted membranes are discontinuous with large gaps or exhibit irregularly multilamellar or indistinct cloud-like morphology (Fig. 2), suggesting that membrane disruption results from structural destabilization likely due to an altered intracellular microenvironment rather than direct interaction between Aβ_{1-42} and lipid bilayers. Furthermore, affected neurons are consistently associated with cytoplasmic acidification. Because numerous autophagy-lysosomal vesicles are dramatically enlarged and retained in affected neurons, once their membranes are compromised, a leakage of their contents will significantly alter the chemical microenvironment of the cytosol causing an intraneuronal chemical lesion. Thus the autophagy-lysosomal injury may be the cause of multiple downstream pathogenic events (Ling & Salvaterra, 2009; Reddy & Beal, 2008).

Mitochondrial deficits are a prominent pathogenic lesion in AD (Moreira et al., 2010a; Moreira et al., 2010b; Reddy & Beal, 2008). Lysosomal-derived chemical lesions may be the proximate cause of these deficits. Electron micrographs show a host of morphological changes including decreased size, abnormal cristae and accumulation of osmiophilic materials in brain tissues from AD patients (Baloyannis, 2006). These morphological features are consistent with our observations using the *Drosophila* model of AD (Ling et al., 2009). Mitochondria provide the energy necessary to support various cellular activities many of which are quite demanding in neurons such as active maintenance of ionic gradients. At the same time, mitochondria also produce free radicals and other oxidative molecules that are intimately involved in the aging process (Balaban et al., 2005). Indeed, metabolic defects, energy deficiency and increased oxidative stress are common pathogenic lesions found in AD (Baloyannis, 2006). In addition to being the major source of intracellular reactive oxygen species, mitochondria are also particularly vulnerable to oxidative damage. Oxidative stress may thus result in a self-amplifying pathogenic lesion. Oxidative stress induces additional compromise of autophagy-lysosomal and mitochondrial membranes and the later will produce more free radicals and further exacerbate the pathogenic lesion.
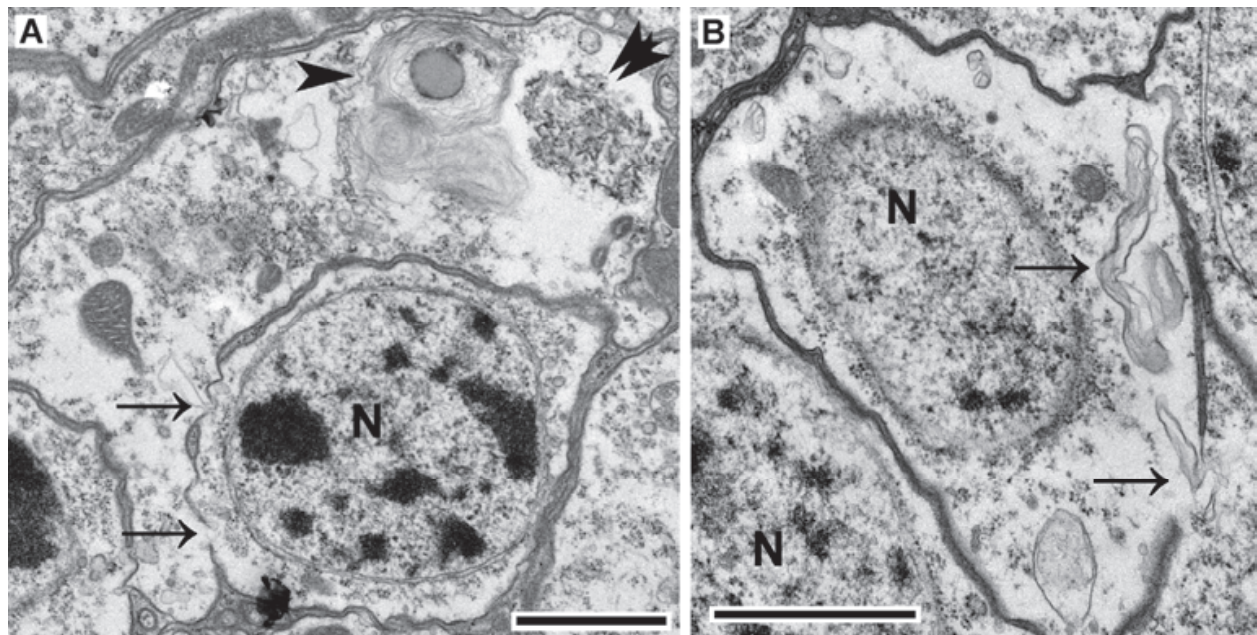
Fig. 2. A$\beta_{1\text{-}42}$ expression causes membrane disruption due to a lysosome-derived chemical lesion. (A) The plasma membranes of an affected and adjacent neuronal somas exhibit discontinuity (arrows). The arrowhead points to a damaged autophagy-lysosomal vesicle; Double arrowheads point to A$\beta_{1\text{-}42}$ aggregate. (B) The plasma and intracellular membranes exhibit multilamellar or cloudy morphology (arrows). N, nuclei. Scale bars = 1µm. The image in (B) was previously published (Ling et al., 2009).

Lysosomal-derived chemical lesions may also destabilize membranes of ER, nuclei and various transport vesicles that will release Ca^{2+} into the cytosol. Neuronal Ca^{2+} is normally stored in membrane compartments such as ER, mitochondria, nuclear envelope and neurotransmitter vesicles (Verkhratsky & Petersen, 1998). Compromise of these membrane-bounded organelles results in a loss of homeostatic intracellular Ca^{2+} control, another prominent chemical lesion in AD pathogenesis (LaFerla, 2002; Supnet & Bezprozvanny, 2010). Cytoplasmic Ca^{2+} is a pivotal neuronal signal regulating multiple intraneuronal activities, neural functions and synaptic plasticity. In vitro application of synthetic Aβ can elevate intracellular Ca^{2+} levels that make cultured neurons more vulnerable to glutamate excitotoxicity (Mattson et al., 1992). Disturbances in neuronal Ca^{2+} may also affect mitochondrial function and vesicular trafficking and, in turn, exacerbate the neurodegenerative cascade.

Lysosomal-derived chemical lesions can destabilize the cytoskeleton, a subcellular component essential for axonal transport, maintenance of normal structure and function of neurites and synapses as well as other cellular activities. Elevated intracellular Ca^{2+} alone was observed to be sufficient to destabilize microtubules and accelerate tau phosphorylation (Mattson et al., 1991), thus linking this chemical lesion with the formation of neurofibrillary tangles. Lysosomal-derived chemical lesions are also associated with the formation of Hirano bodies, rod-shaped and paracrystalline intracellular aggregates composed of actin and cofilin (Maciver & Harrington, 1995). Many neurodegenerative conditions induce the rapid formation of cofilin-actin rod-like inclusions that occur primarily in axons and neurites (Minamide et al., 2000). Cytoskeletal destabilization will disrupt axonal transport of mitochondria and neurotransmitter vesicles as well as many other important subcellular

activities in neurons (McMurray, 2000; Stokin et al., 2005). Tau hyperphosphorylation and microtubule destabilization will also accelerate neurite and synapse atrophy due to the crucial role of microtubules in supporting neuronal terminals and maintaining synaptic integrity (Harada et al., 1994). Thus lysosomal-derived chemical lesions may initiate multiple downstream pathogenic lesions via oxidative stress, Ca^{2+} aberration, cytoplasmic acidification, etc leading to a self-exacerbating and vicious cycle.

Membrane integrity is essential for implementation of neuronal function because the conduction of nerve impulses depends on the maintenance of stable ionic gradients. After an electrical signaling event, restoration of active membrane properties requires an intact membrane to restore proper ionic gradients. Normal neuronal function also relies on the integrity of neurites that extend far from cell bodies. Thus the abnormally elevated Ca^{2+} levels, destabilized microtubules and other cytoskeletal elements, defects in axonal transport, degenerating neurites and synapses resulting from lysosome-derived chemical lesions will cause a decline in neuronal functional performance that may contribute to impairment in the encoding or retrieval of new memories, one of the earliest signs of AD (Selkoe, 2002).

2.2.3 Autophagy-lysosomal injury contributes to neurite and synapse atrophy

Alzheimer's dementia is believed to start from synaptic alterations that correlate more robustly with cognitive decline, memory loss and neurodegeneration than the traditional pathological markers such as plaques and tangles (Selkoe, 2002). Synapse loss and neurite atrophy is critically dependent on cortical Aβ levels. Direct expression of Aβ in *Drosophila* neurons is sufficient to induce synaptic neuropathy (Zhao et al., 2010). However it has never been clear how Aβ induces synapse and neurite damage. Recent evidence demonstrates that neurite atrophy is associated with autophagy activation; and autophagy inhibition protects neurites from degeneration (Wang et al., 2006; Yang et al., 2007). Brain traumatic injury elevates neuronal autophagy and also exhibits axonal degeneration (Chu et al., 2009), supporting an association between the two. Degenerating axons have autophagosome accumulation and cytoplasmic vacuolization along with intracellular Ca^{2+} elevation and cytoskeletal alterations (Knoferle et al., 2010), indicating that lysosomal-derived chemical lesions may contribute to neurite and synapse degeneration. Consistent with this, manipulation of autophagy activity or intracellular Ca^{2+} levels affects the severity of axonal degeneration (Knoferle et al., 2010). In addition, implementation of neuronal function intimately relies on endocytic recycling of neurotransmitters and their receptors at synaptic terminals. Thus subtle changes in the autophagy-lysosomal system may affect synapse construction, maintenance and remodeling (Rowland et al., 2006).

2.2.4 Widespread neuronal loss and autophagy-derived necrosis

A major unanswered question in Alzheimer's pathogenesis is to identify the execution pathway responsible for widespread neuronal death. Apoptosis, a well-controlled and self-regulated programmed cell death, has been widely considered to be the relevant cell death mechanism in many neurodegenerative disorders. However, this appealing mechanism is problematic when applied to Alzheimer's pathogenesis (Graeber & Moran, 2002). Apoptosis is characterized by DNA fragmentation, chromatin condensation, caspase activation, cell shrinking and plasma membrane blebbing. DNA fragmentation detected by the TUNEL method is widespread in AD type neuronal death; however apoptotic morphology is rare

(Jellinger & Stadelmann, 2000). DNA fragmentation, phosphatidylserine exposure on the cell surface as well as mitochondrial dysfunction also exist in other non-apoptotic types of cell death, raising the concern that the widely used TUNEL or annexin V staining alone is not sufficient to validate apoptosis as a particular cell death mechanism.

Autophagy, while generally viewed as a cell survival mechanism, is also thought to cause autophagic cell death (Bursch, 2001), another type of programmed cell death characterized by an abundance of autophagic vesicles in dying cells (Chen et al., 2010). Autophagy over-activation in *Drosophila* larval fat body results in a significant cell loss suggesting that this pathway is capable of inducing cell death (Scott et al., 2007). Neuronal death after hypoxic and ischemic brain injury is also associated with a dramatic increase of autophagic vesicles; furthermore, mice with *Atg7* deficiency show nearly complete protection from neuronal death, suggesting that autophagy plays an essential role in executing neuronal death after hypoxic and ischemic injury (Koike et al., 2008). Cellular models for Parkinson's disease using the 1-methyl-4-phenylpyridium (MPP+) neurotoxin show that induced autophagic toxicity leads to neuronal death (Chu et al., 2007). Even with these observations, it is still controversial whether the presence of autophagy morphology is a cause or a result of cell death.

Either brain aging or $A\beta_{1-42}$ production causes a chronic deterioration of the neuronal autophagy-lysosomal system leading to accumulation of inefficient and enlarged autophagy-lysosomal vesicles in neurons (Ling & Salvaterra, 2011a). Lysosomal compartments are known for membrane permeabilization that release lysosomal cathepsins and other hydrolases into the cytosol; however, the process and the extent of the leakage are usually regulable or may activate a controlled mode of cell death (i.e. apoptosis) (Boya & Kroemer, 2008; Guicciardi et al., 2004). Intriguingly, we found that $A\beta_{1-42}$-induced lysosomal leakage causes uncontrollable intraneuronal necrotic destruction (Ling et al., 2009). Some dying neurons lose their normal cytosolic structures but maintain a relatively normal shape for the plasma membrane forming balloon cells (Fig. 3). Necrotic cell death usually stimulates a powerful inflammatory response. Indeed, neuroinflammation is a prominent pathological feature of AD (Sastre et al., 2011). These data indicate that autophagy-derived necrosis is likely to be the primary cell death execution pathway responsible for the widespread neuronal loss in AD pathogenesis.

2.3 Causative connections between AD risk factors and autophagy-lysosomal injury

The firmly established risk factors of AD are increasing age, the ε4 allele of the apolipoprotein E (*ApoE*) gene, familial history of AD and Down syndrome. Down syndrome-associated AD neuropathology is thought to be a consequence of the over dosage of the *APP* gene. Familial history as a risk factor is particularly associated with early-onset familial AD and is attributable to various inheritance-acquired mutations predominantly located in three genes: *APP*, *PSEN1* and *PSEN2* (Bertram & Tanzi, 2008). The *ApoE* ε4 allele is associated with sporadic AD (Bertram & Tanzi, 2008) and may account for 50% of AD cases in United States (Raber et al., 2004). Thus among the 4 firmly-established AD associated genes, *APP*, *PSEN1* and *PSEN2* are causative genes for familial AD; whereas *ApoE* is a susceptibility gene for sporadic AD. Among various AD risk factors, advancing age is the most prominent as evident by a dramatically increased prevalence of AD as people get older. The incidence of AD in the American population raises from 2% at 65–74 years old to 19% at 75–84 and 42% or more in individuals over 85 years old (see Alzheimer's Disease Facts and Figures 2007, Alzheimer's Association). Besides aging, other less prominent risk factors include traumatic brain injuries, increased cholesterol levels and

other lifestyle and pathophysiological conditions such as high blood pressure, heart disease, stroke and diabetes (Flicker, 2010; Lahiri & Maloney, 2010; McDowell, 2001; Martins et al., 2006; Rosendorff et al., 2007). It is currently unknown how those causative and susceptibility genes, aging, various environmental and lifestyle risk factors interact to affect the disease onset. Here we consider how the autophagy-lysosomal injury establishes a pathological connection between the main etiological factors and AD onset. Other risk factors that could also be attributable to AD pathogenesis through direct or indirect connection to the autophagy-lysosomal injury are not discussed here due to space limitations.
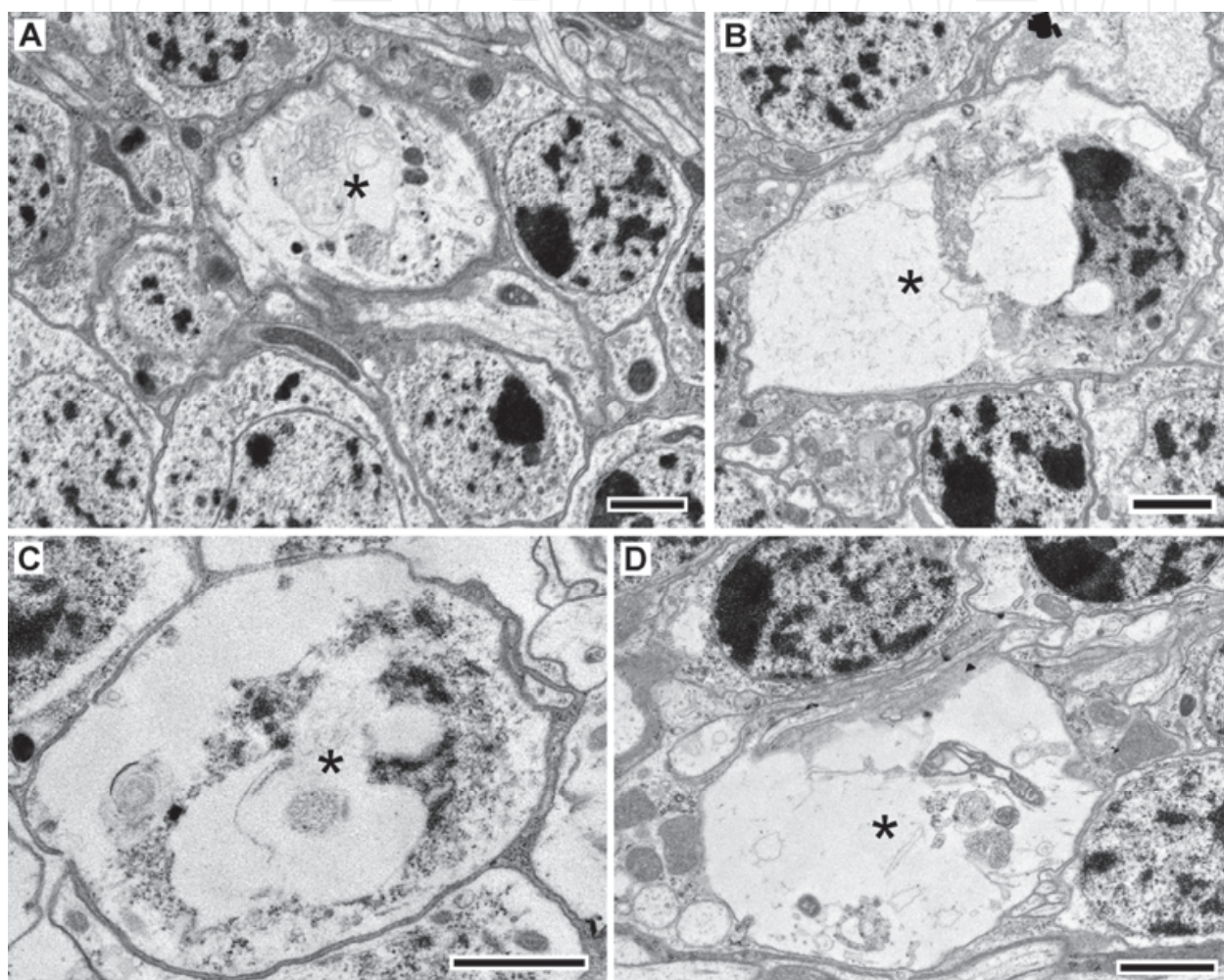
Fig. 3. The morphology of balloon cells results from $A\beta_{1\text{-}42}$-induced neurodegeneration. (A) A balloon cell of degenerated neuronal soma surrounded by relatively normal neuronal somas. (B-C) Necrotic intracellular destruction causes the formation of balloon cells. (D) A balloon cell is electron lucent with partially digested mitochondria and other organelles. Stars (*), balloon cells. Scale bars = 1μm.

2.3.1 Genetic determinants and autophagy-lysosomal Aβ degradation

Amyloid deposition formed by Aβ aggregates is a pathological hallmark of AD. Familial AD-associated mutations on *APP*, *PSEN1* and *PSEN2* genes mostly favor production of hyperaggregatable $A\beta_{1\text{-}42}$ rather than $A\beta_{1\text{-}40}$. More AD susceptibility loci recently identified are also associated with Aβ metabolism (Sleegers et al., 2010). $A\beta_{1\text{-}42}$ in its fibrillary or

oligomeric form is believed to be the main causative agent of AD. Aβ overproduction causes a dementia-like phenotype in transgenic animals (McGowan et al., 2006). Direct Aβ$_{1-42}$ expression in *Drosophila* brains induces age-dependent neurodegeneration (Finelli et al., 2004; Iijima et al., 2004; Crowther et al., 2005; Ling et al., 2009), suggesting that overproduction of Aβ$_{1-42}$ alone is sufficient to initiate neurodegenerative cascade. Even though Aβ$_{1-42}$ is the most widely accepted causative agent for AD, brain amyloid load does not correlate strictly with the severity of dementia. In an interrupted clinical trial, anti-Aβ immuno-therapy resulted in decreased brain amyloidosis but exhibited subtle cognitive benefits (Gilman et al., 2005). Furthermore, Aβ is a normal component of serum and cerebrospinal fluid in individuals with no disease symptoms. These observations complicate the cause-effect relationship between Aβ and AD. However, these seemingly paradoxical aspects of Aβ and AD are compatible with the self-exacerbating autophagy-lysosomal cascade that is initiated by but then independent of further Aβ$_{1-42}$ production as discussed in the next section.

Aβ$_{1-42}$-induced neurodegeneration via an autophagy-lysosomal injury does not conflict with the general protective function of the autophagy-lysosomal machinery. The protective or detrimental effect of neuronal autophagy is primarily dependent on the efficiency of lysosomal degradation of disease-associated aggregate-prone proteins and damaged organelles. Not all aggregate-prone proteins are amenable to autophagic degradation (Wong et al., 2008). Human Aβ$_{1-40}$ and Aβ$_{1-42}$ expressed in *Drosophila* brain have differential effects on neuronal autophagy-lysosomal degradation (Ling et al., 2009). Aβ$_{1-42}$ induces an age-dependent functional defect as well as a structural compromise in autophagy-lysosomal vesicles. These deteriorated vesicles massively accumulate in affected neurons and their size is dramatically enlarged. Aβ$_{1-40}$, in contrast, does not produce any detectible changes in either the neuronal autophagy-lysosomal machinery or neurological defects in animals, suggesting that Aβ$_{1-40}$ may be amenable to autophagic removal and thus lack significant neurotoxicity. The differential autophagic responses to Aβ$_{1-40}$ vs. Aβ$_{1-42}$ is consistent with the paradoxical observations that APP proteolysis primarily generates Aβ$_{1-40}$ (Hartmann et al., 1997), while it is Aβ$_{1-42}$ that predominantly accumulates in neurons (Gouras et al., 2005). The early-onset deterioration of neuronal autophagy-lysosomal machinery specific to Aβ$_{1-42}$ but not Aβ$_{1-40}$ is also consistent with the causative role of Aβ$_{1-42}$ in AD pathogenesis.

2.3.2 The risk factors of ApoE and cholesterol

ApoE and cholesterol, known to have a strong impact on development of cardiovascular disease (Purnell et al., 2009), are also important modifiers of AD onset (Lahiri et al., 2004; Sambamurti et al., 2004). The underlying mechanism linking ApoE and cholesterol with AD pathogenesis is still not completely understood. Cholesterol is a normal membrane component that modifies membrane fluidity. Accumulating evidence shows that cholesterol modulates Aβ production and aggregation through its effect on lipid rafts. Membrane-embedded APP undergoes amyloidogenic proteolysis by beta-secretase (BACE1) or non-amyloidogenic proteolysis by alpha-secretase. Lipid rafts, the cholesterol- and sphingolipid-enriched membrane microdomains (Simons & Toomre, 2000), play an essential role in amyloidogenic APP proteolysis, because the lipid raft enhances accessibility of BACE1 to APP (Ehehalt et al., 2003; Rushworth & Hooper, 2010; Vetrivel & Thinakaran, 2010). Lipid rafts may also facilitate Aβ aggregation (Rushworth & Hooper, 2010) and extracellular Aβ internalization (Lai & McLaurin, 2010). Increased cholesterol accelerates APP localization

into lipid rafts and enhances Aβ generation (Kosicek et al., 2010; Michikawa, 2003); consistent with observations that elevated dietary cholesterol uptake or hypercholesterolemia is associated with increased formation of amyloid plaques (Kivipelto et al., 2001). In addition, cholesterol depletion inhibits neuronal Aβ generation (Sambamurti et al., 2004); and cholesterol-reducing statin drugs appear to reduce the risk of dementia (Gibson Wood et al., 2003).

ApoE is the major carrier of lipids, including cholesterol, in the brain. Lipidated ApoE has been shown to inhibit Aβ transport across blood-brain-barrier and facilitate its degradation (Fan et al., 2009). The ε4 allele of *ApoE* gene was observed to contribute to Aβ deposition (Jones et al., 2011; Raber et al., 2004), favor cerebral amyloid angiopathy (Kumar-Singh, 2008) and promote earlier AD onset (Roses, 1996). So ApoE and cholesterol may affect the onset of AD likely through modification of Aβ production and aggregation and thus indirectly influence the neuronal autophagy-lysosomal machinery. It is also plausible that there is a direct interaction between ApoE/cholesterol and the efficiency of autophagic-lysosomal turnover as a potential mechanism for the altered risk of AD. ApoE/cholesterol modifies membrane fluidity that could directly affect the trafficking of lysosomal vesicles as well as their degradation. ApoE in neurons is actively recycled by endocytosis (DeKroon & Armati, 2001) but not amenable to intracellular degradation (Rensen et al., 2000). ApoE ε4 also appears to accentuate abnormal changes in early endosomes at preclinical stages of AD (Cataldo et al., 2000), impair endocytosis of extracellular Aβ internalization, prevent lysosomal degradation of Aβ (Yamauchi et al., 2002) and increase intracellular Aβ$_{1-42}$ accumulation (Yu et al., 2010; Zerbinatti et al., 2006).

2.3.3 Brain aging and autophagy-lysosomal catabolism

AD exhibits multiple neuropathological signs and clinical symptoms that distinguish it from normal brain aging. However, normal aging brains undergo similar histopathologic changes seen in AD including the presence of plaques, tangles, Hirano bodies, GVD, neurite and synapse deficit, shrinkage in overall brain volume, decreased brain weight and enlargement of brain ventricles (Anderton, 1997; Drachman, 2007). The differences in these changes comparing AD with normal aging appear to be quantitative rather than qualitative (Ball, 1982). Even after a century of intensive studies, the pathogenic connection between normal aging and AD remains elusive.

Human Aβ$_{1-42}$ expression in *Drosophila* brains results in a massive accumulation of enlarged dysfunctional autophagy–lysosomal vesicles that become increasingly compromised with age leading to deterioration of neuronal integrity and necrotic intraneuronal destruction (Ling et al., 2009). Intriguingly, the process of normal aging undergoes similar pathogenic changes in wild-type *Drosophila* brains without expression of any disease-associated proteins (Ling & Salvaterra, 2011a). The only difference between Aβ$_{1-42}$ expression and normal brain aging is the time scale of the neuropathological progression. Aβ$_{1-42}$ induces an early-onset autophagy-lysosomal neuropathology which progresses rapidly; whereas normal aging has a late-onset neuropathology which progresses at a slower rate. These data are consistent with observations that low levels of abnormal autophagy-lysosomal vesicles, characterized as typical granulovacuolar degeneration, are also observed in hippocampal neurons from brains of mentally normal patients (Ball & Lo, 1977), suggesting that brains normally undergo deterioration of the autophagy-lysosomal machinery during aging. Thus normal brain aging accompanies neurodegeneration via an autophagy-lysosomal neuropathology

that may occur at a slow enough rate or on a small enough scale. Any cognitive decline associated with normal aging-associated neurodegeneration will go unnoticed. Consistent with this possibility, individuals with normally measured cognitive function undergo an age-dependent reduction in overall brain volume and weight as well as an age-dependent enlargement of brain ventricles due to neuron loss (Anderton, 1997).

Autophagy-lysosomal machinery maintains intracellular homeostasis and thus protects neurons from degeneration. Basal levels of neuronal autophagy are believed to decrease with age (Komatsu et al., 2007); however, direct evidence supporting this view is absent. In *Drosophila* brains autophagy activity during normal aging appears to be stable based on observations that no significant changes occurs in expression levels for several autophagy-related genes (Ling & Salvaterra, 2011b). Moreover, induction of neuronal autophagy in a conditional *Drosophila* model is protective in young animals, but likely detrimental in older animals (Ling & Salvaterra, 2011a). Therefore it is reasonable to propose that the autophagy–lysosomal machinery likely shifts from a functional and protective status to a pathological and deleterious status during brain aging. Consistent with this, autophagic function is known to decline with age (Bergamini et al., 2007). Taken together, either brain aging or $A\beta_{1-42}$ proteotoxicity contributes to the chronic deterioration of the neuronal autophagy-lysosomal system. The deterioration of this catabolic machinery appears to be a key pathogenic event that converts normal brain aging into pathological aging leading to Alzheimer's neurodegeneration.

3. Autophagy-lysosomal cascade: A hypothesis for AD pathogenesis

Remarkable progress has been made in studying many aspects of AD. Unfortunately, this has not resulted in the successful development of effective treatments, primarily because of the absence of a definite pathogenic mechanism. Numerous hypotheses have been advanced to address AD pathogenesis including the amyloid cascade, membrane disruption/$A\beta$ ion channel, mitochondrial abnormalities, energy deficits, glutamate excitotoxicity, cerebrovascular dysfunction, neuroinflammation, oxidative stress, Ca^{2+} dyshomeostasis and cytoskeletal aberrations. Each of these ideas were proposed and developed based on one or few particular pathological features of AD. As a consequence most of the currently favored hypotheses provide only a limited view rather than a more global perspective of the pathogenic mechanism. It also remains unclear what initial event(s) trigger the pathogenic cascade and how so many different pathological insults can be attributed to the key pathogenic event.

Extensive autophagy involvement in AD has been well documented (Nixon et al., 2005; Shacka et al., 2008; Suzuki & Terry, 1967). However, it remains unsettled if autophagy plays a causative role, a protective role or is a consequence of the disease process itself (Ling & Salvaterra, 2009). Among the various signs and lesions of AD neuropathology, compromised autophagy-lysosomal vesicles and their resultant injuries appear to play a central role in initiating the pathogenic cascade leading to disease progression. Based on *Drosophila* models of AD and brain aging as well as growing evidence in this field, we have proposed an autophagy-derived neurodegenerative cascade initiated by $A\beta_{1-42}$ and enhanced by aging (Ling & Salvaterra, 2009, 2011a; Ling et al., 2009).

APP proteolysis and $A\beta$ production occurs at membrane surfaces facing the lumen of membrane compartments including ER, Golgi apparatus and endosomal vesicles (Fiala, 2007; Gouras et al., 2005). $A\beta$ is constitutively produced in human brains throughout the normal

lifespan. Apparently the levels of newly generated Aβ peptides may not be sufficient to initiate a pathogenic cascade in healthy neurons; however, due to their amphipathic property, they may disturb local membranes and the functional execution of host organelles. These organelles, if damaged, will be sequestered by autophagy. However, Aβ particularly $A\beta_{1-42}$ cannot be efficiently degraded in autophagy-lysosomal vesicles especially under chronic deterioration of this machinery during advancing age (Ling & Salvaterra, 2011a). Other indigestible proteins and lipids (for example lipofuscin) may synergistically contribute to the deterioration of autophagy-lysosomal machinery causing cargo storage in enlarged vesicles. Consistent with this view, intracellular Aβ peptides predominantly accumulate within autophagic and endosomal vesicles (Nixon, 2004; Takahashi et al., 2002; Yu et al., 2005) and AD-like neuropathological phenotypes are also seen in some lysosomal storage diseases (Bahr & Bendiske, 2002; Ohm et al., 2003; Jin et al., 2004; Settembre et al., 2008).

Numerous lysosomal vesicles in cytosol would represent a large source of acidic contents and lysosomal hydrolases. The enlarged size and long-term retention of these vesicles may make them easily compromised especially when $A\beta_{1-42}$ becomes concentrated within them. Compromised vesicles result in leakage of their acidic contents into cytosol. This will destabilize other intracellular structures and organelles including ER and mitochondria leading to oxidative stress and Ca^{2+} dyshomeostasis. The resultant damage from this altered intracellular microenvironment will further activate autophagy causing additional pathogenic stress. Thus a self-exacerbated pathogenic cascade is formed through initiation, dysfunction, compromise of autophagic vesicles and the resultant cytosolic chemical lesions. This neurodegenerative cascade is initiated by $A\beta_{1-42}$ and enhanced by aging and eventually results in necrotic neuronal death. Once initiated, the cascade would likely become independent of continuous Aβ production since cytosolic chemical lesions would drive it as a progressive and irreversible pathogenic pathway. This autophagy-lysosomal-derived neurodegenerative cascade provides a common cellular framework for a detailed mechanistic understanding of the heterogeneous aspects of AD neuropathology as the signs, the lesions and the causes of the disease.

4. Conclusion

Alzheimer's disease is an incurable terminal neurodegenerative disorder with multifactorial etiology and heterogeneous pathology. The clearer we understand the pathogenic mechanism(s) regarding its causes, lesions and signs, the better we should be able to develop effective treatments for mitigating or even preventing this disastrous disorder. The autophagy-lysosomal system, a bulk process for removal of intracellular toxic proteins and damaged organelles, appears to play a central role in the disease pathogenesis. Based on our recent work and a large volume of previous studies from other groups, we propose an autophagy-lysosomal cascade that is attributable to various AD etiologies, and responsible for the hierarchical pathological signs and pathogenic lesions. One of the prominent features of this pathogenic mechanism is its potential for self-exacerbation. Once progressing to an uncontrollable stage, this cascade is likely to be independent of initial contributions from causative factors and will continue to develop progressively and irreversibly. This feature fits well with the onset of pathological and clinical AD. It has never been clear when the disease pathology actually starts; however, once diagnosed, the disease develops progressively and relentlessly. This feature emphasizes the importance of preventative strategies applied to the at-risk individuals prior to the actual occurrence of this disease.

The autophagy-lysosomal cascade for AD pathogenesis appears to provide a unified cellular framework for understanding the disease; however, therapeutic development targeting autophagy-lysosomal pathway is far from maturation. Our knowledge of the autophagy-lysosomal system is fast growing (Klionsky, 2007). Many basic aspects of the pathway are still waiting for detailed characterization. A beneficial outcome from manipulation of autophagy activity under neurodegenerative conditions is still uncertain. Even though basal autophagy is protective and autophagy induction has prosurvival effects observed in some disease models (Rubinsztein et al., 2007), detrimental effects of increased autophagy are also associated with certain pathological conditions (Cherra et al., 2010; White & DiPaola, 2009). Our studies, however, emphasize that enhancing the maintenance of an integrated and efficient autophagy–lysosomal system in brain rather than simply induction of autophagy activity would be a promising therapeutic direction for anti-aging or prevention of AD.

5. Acknowledgements

6. References

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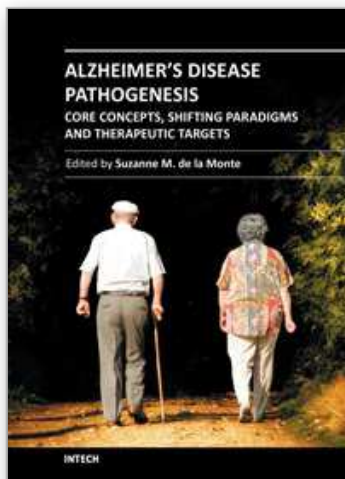
Alzheimer's Disease Pathogenesis-Core Concepts, Shifting Paradigms and Therapeutic Targets

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Alzheimer's Disease Pathogenesis: Core Concepts, Shifting Paradigms, and Therapeutic Targets, delivers the concepts embodied within its title. This exciting book presents the full array of theories about the causes of Alzheimer's, including fresh concepts that have gained ground among both professionals and the lay public. Acknowledged experts provide highly informative yet critical reviews of the factors that most likely contribute to Alzheimer's, including genetics, metabolic deficiencies, oxidative stress, and possibly environmental exposures. Evidence that Alzheimer's resembles a brain form of diabetes is discussed from different perspectives, ranging from disease mechanisms to therapeutics. This book is further energized by discussions of how neurotransmitter deficits, neuro-inflammation, and oxidative stress impair neuronal plasticity and contribute to Alzheimer's neurodegeneration. The diversity of topics presented in just the right depth will interest clinicians and researchers alike. This book inspires confidence that effective treatments could be developed based upon the expanding list of potential therapeutic targets.

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