



Review

Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology

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ABSTRACT

Mitochondria are uniquely poised to play a pivotal role in neuronal cell survival or death because they are regulators of both energy metabolism and cell death pathways. Extensive literature exists supporting a role for mitochondrial dysfunction and oxidative damage in the pathogenesis of Alzheimer's disease. This review discusses evidence indicating that mitochondrial dysfunction has an early and preponderant role in Alzheimer's disease. Furthermore, the link between mitochondrial dysfunction and autophagy in Alzheimer's disease is also discussed. As a result of insufficient digestion of oxidatively damaged macromolecules and organelles by autophagy, neurons progressively accumulate lipofuscin that could exacerbate neuronal dysfunction. Since autophagy is the major pathway involved in the degradation of protein aggregates and defective organelles, an intense interest in developing autophagy-related therapies is growing among the scientific community. The final part of this review is devoted to discuss autophagy as a potential target of therapeutic interventions in Alzheimer's disease pathophysiology.

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1. Introduction

Alzheimer's disease (AD) is the most common form of dementia and affects millions of people worldwide. The disorder is characterized by severe memory loss, with episodic memory being particularly impaired during the initial phases. Most AD cases occur sporadically, although inheritance of certain susceptibility genes enhances the risk. Familial AD represents the minority of AD cases and is caused by mutations in genes encoding for either the amyloid β precursor protein (A β PP), presenilin 1 (PS1), or presenilin 2 (PS2). Two pathological hallmarks are observed in AD brains at autopsy: intracellular neurofibrillary tangles (NFTs) and extracellular senile plaques (SP) in the neocortex, hippocampus, and other subcortical regions essential for cognitive function. NFTs are formed from paired helical filaments composed of neurofilaments and hyperphosphorylated tau protein. In turn, plaque cores are formed mostly from deposition of amyloid β (A β) peptide that results from the cleavage of the A β PP.

The literature shows that mitochondrial dysfunction and oxidative stress play an important role in the early pathology of AD [1–4]. Indeed, there are strong indications that oxidative stress occurs prior to the onset of symptoms in AD and oxidative damage is found not only in the vulnerable regions of the brain affected in disease [5–7] but also peripherally [8–11]. Moreover, it has been shown that oxidative damage occurs before A β plaque formation [5] supporting a causative

role of mitochondrial dysfunction and oxidative stress in AD. This review is mainly devoted to discuss evidence showing that mitochondrial dysfunction and oxidative stress is intimately involved in AD pathogenesis. Furthermore, the interplay between mitochondrial abnormalities and autophagy in AD will be addressed. Finally, we will briefly discuss autophagy as a potential target of therapeutic interventions in AD pathophysiology.

2. The good and the bad side of brain mitochondria

Although the brain represents only 2% of the body weight, it receives 15% of cardiac output and accounts for 20% of total body oxygen consumption. This energy requirement is largely driven by neuronal demand for energy to maintain ion gradients across the plasma membrane that is critical for the generation of action potentials. This intense energy requirement is continuous; even brief periods of oxygen or glucose deprivation result in neuronal death.

Mitochondria are increasingly recognized as subcellular organelles that are essential for generating the energy that fuels normal cellular function while, at the same time, they monitor cellular health in order to make a rapid decision (if necessary) to initiate programmed cell death (Fig. 1). As such, the mitochondria sit at a strategic position in the hierarchy of cellular organelles to continue the healthy life of the cell or to terminate it. Mitochondria are essential for neuronal function because the limited glycolytic capacity of these cells makes them highly dependent on aerobic oxidative phosphorylation (OXPHOS) for their energetic needs. However, OXPHOS is a major

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source of endogenous toxic free radicals, including hydrogen peroxide (H_2O_2), hydroxyl ($\text{HO}\cdot$) and superoxide ($\text{O}_2^{\cdot-}$) radicals that are products of normal cellular respiration (Fig. 1).

With inhibition of electron transport chain (ETC), electrons accumulate in complex I and coenzyme Q, where they can be donated directly to molecular oxygen to give $\text{O}_2^{\cdot-}$ that can be detoxified by the mitochondrial manganese superoxide dismutase (MnSOD) to give H_2O_2 that, in turn, can be converted to H_2O by glutathione peroxidase (GPx). However, $\text{O}_2^{\cdot-}$ in the presence of nitric oxide ($\text{NO}\cdot$), formed during the conversion of arginine to citrulline by nitric oxide synthase (NOS), can lead to peroxynitrite (ONOO^-). Furthermore, H_2O_2 in the presence of reduced transition metals can be converted to toxic $\text{HO}\cdot$ via Fenton and/or Haber Weiss reactions. Several efficient enzymatic processes are continuously operational to quench the reactive species including SOD, GPx, superoxide reductase (SRed), catalase (CAT), peroxiredoxin (Prx), thioredoxin/thioredoxin reductase (Trx/TrxRed) [12]. Inevitably, if the amount of free radical species produced overwhelms the neuronal capacity to neutralize them, oxidative stress occurs, followed by mitochondrial dysfunction and neuronal damage. Reactive species generated by mitochondria have several cellular targets including mitochondrial components themselves (lipids, proteins, and DNA). The lack of histones in mitochondrial DNA

(mtDNA) and the diminished capacity for DNA repair render the mitochondria an easy target to oxidative stress events.

The central nervous system (CNS) is particularly susceptible to reactive species-induced damage [13] because (1) it has a high consumption of oxygen, (2) it contains high levels of membrane polyunsaturated fatty acids susceptible to free radical attack, (3) it is relatively deficient in oxidative defenses (poor CAT activity and moderate SOD and GPx activities), and (4) a high content in iron and ascorbate can be found in some regions of the CNS, enabling generation of more reactive species through the Fenton/Haber Weiss reactions.

Mitochondria also serve as high-capacity Ca^{2+} sinks, which allows them to stay in tune with changes in cytosolic Ca^{2+} loads and aid in maintaining cellular Ca^{2+} homeostasis that is required for normal neuronal function [14]. Conversely, excessive Ca^{2+} uptake into mitochondria has been shown to increase ROS production, inhibit ATP synthesis, induce mitochondrial permeability transition pore (PTP), and release small proteins that trigger the initiation of apoptosis, such as cytochrome c and apoptosis-inducing factor (AIF), from the mitochondrial intermembrane space into the cytoplasm. Released cytochrome c binds apoptotic protease-activating factor 1 (Apaf-1) and activates the caspase cascade [15]. Such alterations in

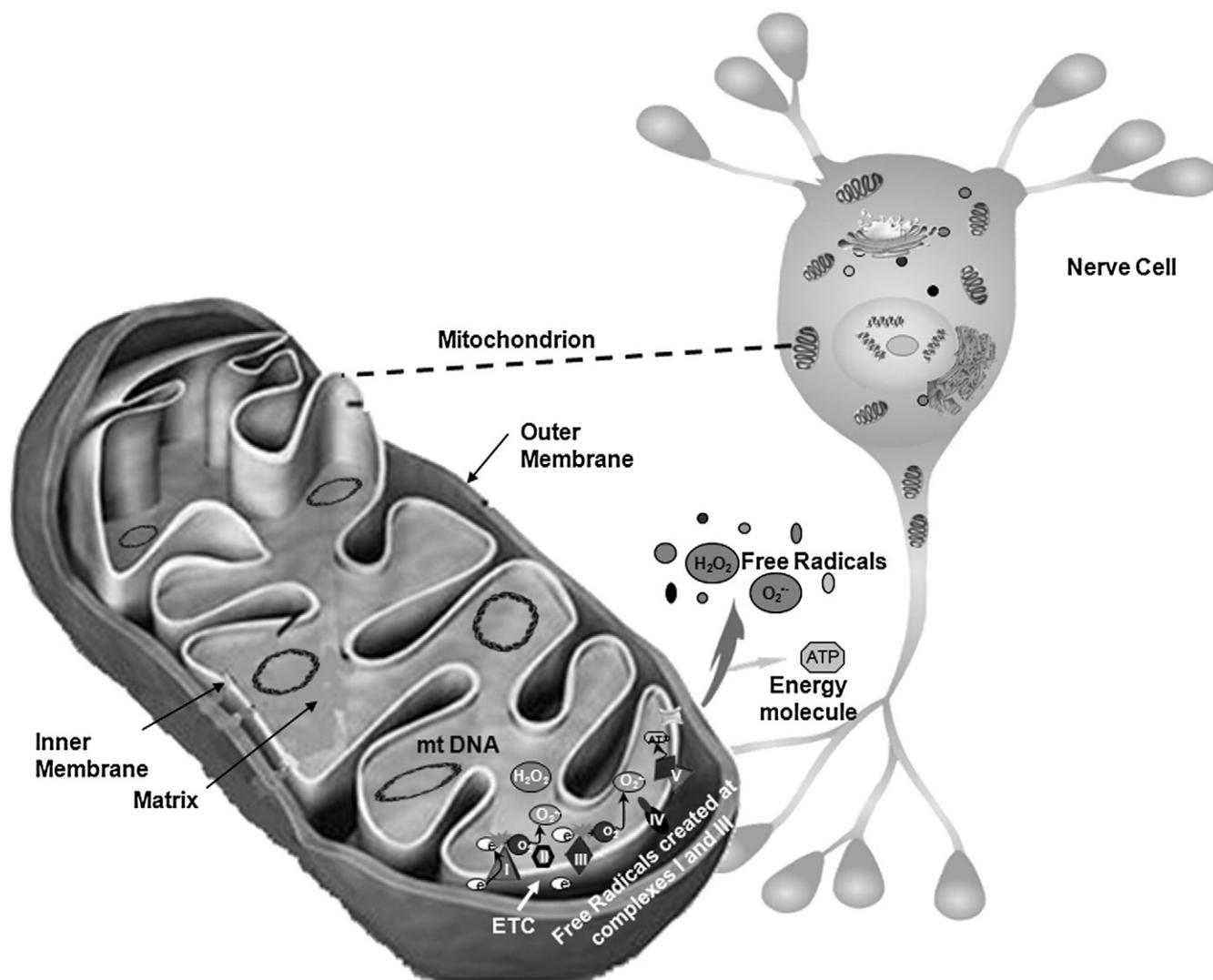


Fig. 1. Dual role of mitochondria. Besides the fundamental role of mitochondria in the generation of energy (ATP), these organelles are also the main producers of oxygen free radicals. If the defense mechanisms are debilitated these reactive species initiate a cascade of deleterious events within the cell. See text for more detail. ETC, electron transport chain; H_2O_2 , hydrogen peroxide; mtDNA, mitochondrial DNA; $\text{O}_2^{\cdot-}$, superoxide anion radical; e^- , electrons; O_2 , molecular oxygen.

mitochondrial function have been proposed as a causative mechanism in the pathogenesis of AD (Fig. 2).

3. Mitochondrial dysfunction is an early event in AD

Swerdlow and Khan [16,17] proposed the mitochondrial cascade hypothesis to explain late-onset, sporadic AD. In short, this hypothesis states that, in the sporadic AD, mitochondrial dysfunction is the primary event that causes A β deposition, synaptic degeneration, and NFTs formation. Indeed, there is a bulk of studies showing that mitochondrial dysfunction is a common event in AD.

Using in situ hybridization to mtDNA, immunocytochemistry of cytochrome oxidase (COX), and morphometry of electron micrographs of biopsy specimens, we demonstrated that neurons showing increased oxidative damage in AD also possessed a striking and significant increase in mtDNA and COX [18,19]. Moreover, we found that much of the mtDNA and COX was localized in the neuronal cytoplasm and, in the case of mtDNA, in vacuoles associated with lipofuscin, whereas morphometric analysis showed that mitochondria were significantly reduced in AD. Interestingly, the cellular expression

of COX subunits II and IV is reduced during aging and these age-related changes are more marked in AD [20], suggesting that aging is a major risk factor for this disease. However, Cottrell et al. [21] observed that the distribution of amyloid plaques is anatomically distinct from the COX-deficient hippocampal pyramidal neurons, and the neurons containing NFT or apoptotic labeling were always COX-positive. The authors concluded that COX-deficient, succinate dehydrogenase-positive hippocampal neurons indicative of high mtDNA mutation load do not appear to be prone to apoptosis or to directly participate in the overproduction of tau or A β [21].

Wang et al. [22] quantified multiple oxidized bases in nuclear DNA and mtDNA of frontal, parietal, and temporal lobes and cerebellum from short postmortem interval AD brain and age-matched control subjects using gas chromatography/mass spectrometry with selective ion monitoring and stable labeled internal standards. It was found that levels of multiple oxidized bases in AD brain specimens were significantly higher in frontal, parietal, and temporal lobes compared to control subjects and that mtDNA had approximately 10-fold higher levels of oxidized bases than nuclear DNA. These data are consistent with higher levels of oxidative stress in mitochondria. Furthermore,

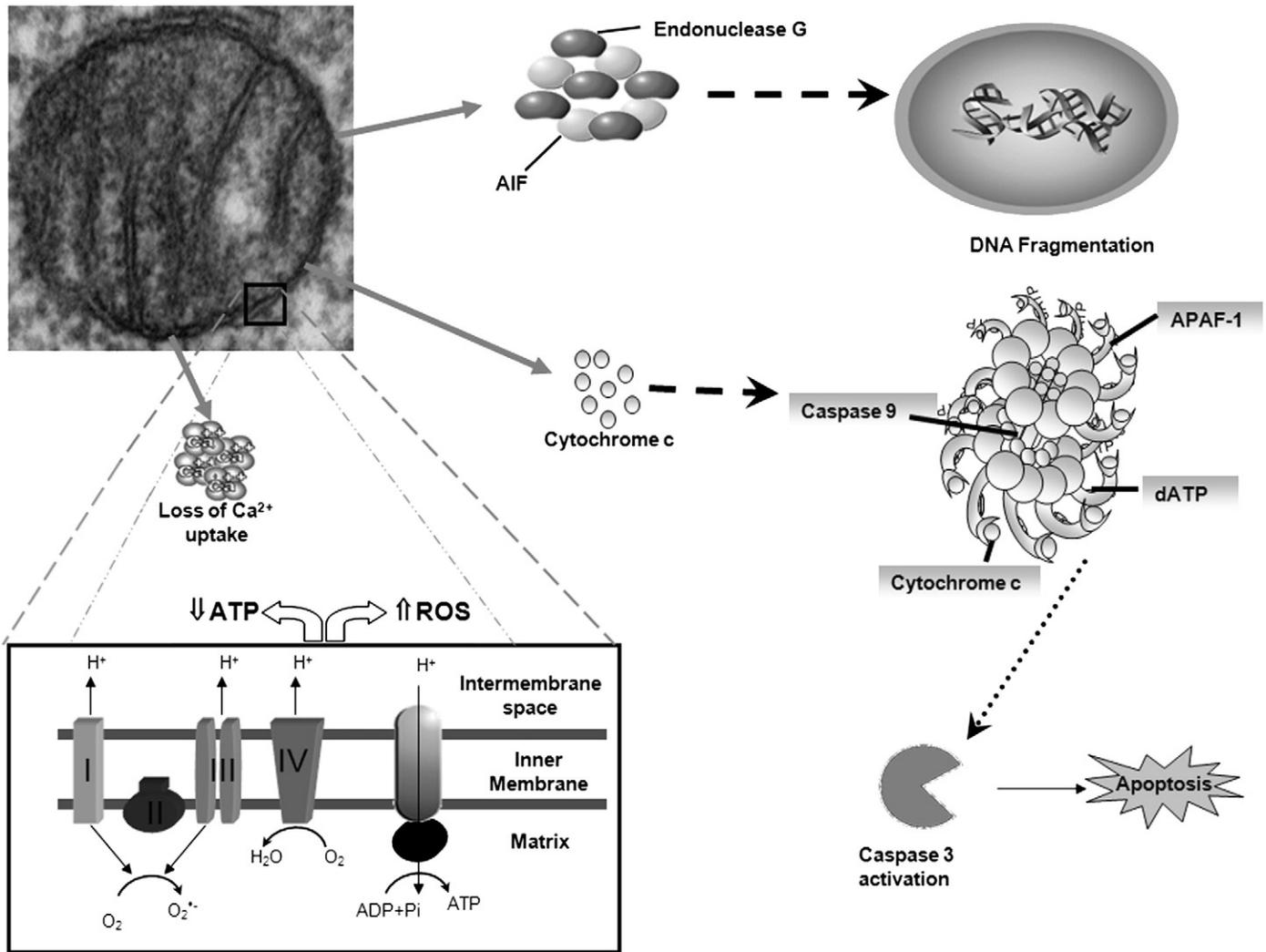


Fig. 2. Mitochondrial dysfunction in Alzheimer's disease. Mitochondrial abnormalities associated with enhanced oxidative stress have long been recognized to play a major role in cell degeneration and death that occur in Alzheimer's disease. Indeed, during Alzheimer's disease evolution, mitochondria suffer profound alterations that lead to a reduced generation of adenosine triphosphate (ATP) and enhanced production of reactive oxygen species (ROS). Mitochondria also lose calcium (Ca²⁺) buffering capacity, which can initiate in a cascade of deleterious within the cell. Impaired mitochondria also release several pro-apoptotic factors upon induction of apoptosis. These factors are either directly triggering apoptosis by associating with cytosolic factors to form the apoptosome. Finally, some mitochondrial, pro-apoptotic proteins translocate into the nucleus to induce deoxyribonucleic acid (DNA) fragmentation. Altogether, these mitochondrial alterations contribute to cell degeneration and death. AIF, apoptosis-inducing factor; ApAF-1, protease-activating factor 1; dATP, 2'-deoxyadenosine 5'-triphosphate; ROS, reactive oxygen species; ADP, adenosine diphosphate; H⁺, protons; H₂O, water; Pi, inorganic phosphate; O₂, molecular oxygen; O₂^{-•}, superoxide anion radical.

8-hydroxydeoxyguanosine (8-OHG) was approximately 10-fold higher than other oxidized base adducts in both AD and control subjects. DNA from temporal lobe showed the most oxidative damage, whereas cerebellum was only slightly affected in AD brains [22]. Altogether, these results suggest that oxidative damage to mtDNA may contribute to the neurodegeneration of AD. More recently, Baloyannis [23] reported that, in AD cases, mitochondrial pathology correlates substantially with the dystrophic dendrites, the loss of dendritic branches, and the pathological alteration of the dendritic spines.

The mitochondrial abnormalities observed in the brains of AD subjects are corroborated by animal studies. Indeed, Hauptmann et al. [24] reported that mitochondrial dysfunction is an early event in mice bearing the human Swedish and London mutations and these mitochondrial defects accumulate with age. Recently, Fu et al. [25] also reported that aging potentiates the mitochondrial abnormalities occurring in PS1 transgenic (Tg) mice. Previous studies also demonstrated that the mitochondrial abnormalities appear to be key features during the maturation of AD-like pathology in YAC and C57B6/SJL Tg mice. A higher degree of amyloid deposition, overexpression of oxidative stress markers, mtDNA deletion, and mitochondrial structural abnormalities in the vascular walls of YAC and C57B6/SJL Tg mice was observed when compared to age-matched controls [26,27].

Furthermore, Devi et al. [28] reported that A β PP, due to its chimeric NH₂-terminal signal, is targeted to cortical neuronal mitochondria in a Tg mouse model of AD and the accumulation of full-length A β PP in the mitochondrial compartment in a membrane-arrested form causes mitochondrial dysfunction and impaired energy metabolism. More recently, the same group reported that A β PP accumulates exclusively in the protein import channels of mitochondria of human AD brains [29]. The accumulation of A β PP across mitochondrial import channels inhibits the entry of nuclear-encoded COX subunits IV and Vb proteins, which decreases COX activity and increases H₂O₂ levels. The levels of translocationally arrested mitochondrial A β PP directly correlate with mitochondrial dysfunction. Moreover, apolipoprotein genotype analysis revealed that AD subjects with the E3/E4 alleles have the highest content of mitochondrial A β PP [29].

It has been shown that cultured neurons isolated from Tg mice that overexpress a mutant form of A β PP and A β -binding alcohol dehydrogenase (ABAD) (Tg mA β PP/ABAD) display reduced levels of brain ATP and COX activity, diminished glucose utilization, as well as electrophysiological abnormalities in hippocampal slices compared with Tg mA β PP mice [29]. In contrast, neither Tg ABAD mice nor non-Tg littermates showed similar changes in ATP, COX activity, glucose utilization, or electrophysiological properties [30]. The same authors also reported that cultured neurons isolated from Tg mA β PP/ABAD display spontaneous generation of H₂O₂ and O₂⁻, decreased ATP, release of cytochrome c, and induction of caspase 3-like activity followed by DNA fragmentation and loss of cell viability. Furthermore, generation of ROS is associated with dysfunction at the level of COX [30]. These findings link ABAD-induced oxidant stress to critical aspects of AD-associated cellular dysfunction, suggesting a pivotal role for this enzyme in the pathogenesis of AD.

Takuma et al. [31] performed a comparative study using mice deficient in caspase 3 versus wild-type mice. They microinjected A β _{1–40} into hippocampal regions of the brain of adult mice and found a significant cellular loss in the hippocampal regions of wild-type mice and a dramatic rescue of neuronal cell death in caspase 3-deficient mice, with gene dosage effect. Furthermore, they observed that A β induced a small amount of cell death in cultured neurons prepared from fetal brain of caspase 3-deficient mice; however, cells from wild-type mice suffered a drastic decrease in cell viability. These results suggest that A β -induced neuronal death is mediated by caspase 3 apoptotic cascade. Kaminsky and Kosenko [32] investigated the *in vivo* effects of A β peptides on mitochondrial and non-mitochondrial enzymatic sources of ROS and antioxidant enzymes in rat brain. The

authors observed that the continuous intracerebroventricular infusion of both A β _{25–35} and A β ₄₀ for up to 14 days stimulated H₂O₂ generation in isolated neocortex mitochondria. Infusion of A β ₄₀ led to an increase in MnSOD activity and a decrease in activities of CAT and GPx in mitochondria and to an increase in Cu/ZnSOD and aldehyde oxidase activities and promoted the conversion of xanthine dehydrogenase to xanthine oxidase leading to an increase in the rate of H₂O₂ formation in the cytosol [32]. Resende et al. [33] reported that the triple Tg mouse model of AD present decreased levels of GSH and vitamin E and increased levels of lipid peroxidation. Additionally, the authors observed an increased activity of the antioxidant enzymes GPx and SOD [33]. These alterations are evident during the A β oligomerization period, before the appearance of A β plaques and NFTs, supporting the view that oxidative stress occurs early in the development of the disease. David et al. [34] observed that Tg mice overexpressing the P301 L mutant human tau protein present alterations of metabolism-related proteins including mitochondrial respiratory chain complex components, antioxidant enzymes, and synaptic proteins that are associated with increased oxidative stress. Furthermore, the authors observed that mitochondria from these Tg mice displayed increased vulnerability toward A β insult, suggesting a synergistic action of tau and A β pathology on the mitochondria. The authors suggest that tau pathology involves a mitochondrial and oxidative stress disorder possibly distinct from that caused by A β [34].

A panoply of *in vitro* studies corroborates the idea that mitochondria are key players in AD. It has been previously shown that A β requires functional mitochondria to induce toxicity [35]. Furthermore, Hansson et al. [36] identified an active γ -secretase complex in rat brain mitochondria. Being composed by nicastrin (NCT), anterior pharynx-defective-1 (APH-1), and presenilin enhancer protein 2 (PEN2), this γ -secretase complex cleaves, among other substrates, A β PP generating A β and A β PP intracellular domain. Furthermore, the presence of A β PP was detected in mitochondrial membranes of PC12 cells bearing the Swedish double mutation in A β PP gene [37]. Altogether, these studies placed mitochondria in a privileged position concerning A β PP processing and answered the “old question” of how A β interacts with mitochondria.

Mitochondria continuously undergo two opposing processes: fission and fusion. The disruption of this dynamic equilibrium may herald cell injury or death and may contribute to neurodegenerative disorders. Recently, Wang et al. [38] reported that sporadic AD fibroblasts present alterations in mitochondrial morphology and distribution. They also demonstrated that the mitochondrial abnormalities are due to a decrease in dynamin-like protein 1 (DLP1), a regulator of mitochondrial fission and distribution. The same authors also demonstrated that elevated oxidative stress and increased A β production are likely the potential pathogenic factors that cause DLP1 reduction and abnormal mitochondrial distribution in AD cells [38]. The same group investigated the effect of A β PP and A β on mitochondrial dynamics in neurons [39]. Confocal and electron microscopic analysis demonstrated that approximately 40% M17 cells overexpressing WT APP (A β PPwt M17 cells) and more than 80% M17 cells overexpressing APPsw mutant (A β PPsw M17 cells) displayed alterations in mitochondrial morphology and distribution. These mitochondrial changes were abolished by treatment with β -site A β PP-cleaving enzyme inhibitor IV. From a functional perspective, A β PP overexpression affected mitochondria at multiple levels, including elevating ROS levels, decreasing mitochondrial membrane potential, and reducing ATP production, and also caused neuronal dysfunction such as differentiation deficiency upon retinoic acid treatment. At the molecular level, levels of DLP1 and OPA1 were significantly decreased whereas levels of Fis1 were significantly increased in A β PPwt and A β PPsw M17 cells. Notably, overexpression of DLP1 in these cells rescued the abnormal mitochondrial distribution and differentiation deficiency but failed to rescue mitochondrial fragmentation and functional parameters, whereas overexpression of

OPA1 rescued mitochondrial fragmentation and functional parameters but failed to restore normal mitochondrial distribution. Overexpression of A β PP or A β -derived diffusible ligand treatment also led to mitochondrial fragmentation and reduced mitochondrial coverage in neuronal processes in differentiated primary hippocampal neurons. The authors concluded that A β PP, through A β production, causes an imbalance of mitochondrial fission/fusion that results in mitochondrial fragmentation and abnormal distribution, which contributes to mitochondrial and neuronal dysfunction [39]. More recently, Cho et al. [40] found that NO⁻ produced in response to A β triggered mitochondrial fission, synaptic loss, and neuronal damage, in part via S-nitrosylation of DLP1 (forming SNO-DLP1). Preventing nitrosylation of DLP1 by cysteine mutation abrogated these neurotoxic events. SNO-DLP1 is increased in brains of human AD patients and may thus contribute to the pathogenesis of neurodegeneration.

Previous *in vitro* studies also showed that A β peptides in the presence of Ca²⁺ exacerbate the opening of mitochondrial PTP [41,42]. The PTP induction, a phenomenon characterized by a sudden increase in the permeability of the inner mitochondrial membrane, plays a key role in apoptotic cell death by facilitating the release of apoptogenic factors. It was observed that A β in the presence of Ca²⁺ decreased the mitochondrial transmembrane potential and the capacity of brain mitochondria to accumulate Ca²⁺ and induced a complete uncoupling of respiration and an alteration of the ultrastructural morphology of mitochondria characterized by swelling and disruption of mitochondria cristae [41,42]. Altogether, these results suggest a clear association between A β , mitochondrial dysfunction, and alteration of Ca²⁺ homeostasis. Additionally diabetes-related mitochondrial dysfunction is exacerbated by aging and/or by the presence of A β , supporting the idea that diabetes and aging are risk factors for the neurodegeneration induced by this peptide [43–45]. Aging of diabetic mice induced an impairment of the respiratory chain and a decrease on oxidative phosphorylation efficiency and in the mitochondrial capacity to accumulate Ca²⁺. In the presence of A β _{25–35} or A β ₄₀, the age-related mitochondrial effects are potentiated [43]. Furthermore, we also observed that brain mitochondria isolated from diabetic rats in the presence of A β ₄₀ produce higher levels of H₂O₂ [45]. However, insulin and coenzyme Q10 (CoQ10) treatments prevent the decline in mitochondrial OXPHOS efficiency and avoid an increase in oxidative stress induced by A β [44].

Other studies from our laboratory also showed that pheochromocytoma cells (PC12) exposed to A β ₄₀ and A β _{25–35} present mitochondrial dysfunction characterized by the inhibition of complexes I, III, and IV of the mitochondrial respiratory chain [46]. Recently, Rhein et al. [47] evaluated the mitochondrial respiratory functions and energy metabolism in control and in human wild-type A β PP stably transfected SH-SY5Y cells. The authors observed that complex IV activity was significantly reduced in A β PP cells. In contrast, a significant increase in the activity of complex III was observed. The authors interpreted this increase as a compensatory response in order to balance the defect of complex IV. However, this compensatory mechanism did not prevent the strong impairment of total respiration in A β PP cells. As a result, the respiration together with ATP production decreased in the A β PP cells in comparison with the control cells [47]. A study from our laboratory also shows that AD fibroblasts present high levels of oxidative stress and apoptotic markers when compared with young and age-matched controls [2]. Furthermore, AD-type changes could be generated in control fibroblasts using *N*-methylprotoporphyrin to inhibit COX assembly indicating that the observed oxidative damage was associated with mitochondrial dysfunction. The effects of *N*-methylprotoporphyrin were reversed or attenuated by both lipoic acid (LA) and *N*-acetyl cysteine (NAC) [11]. These results suggest that mitochondria are important players in oxidative damage that occurs in AD and that antioxidant therapies may be promising.

Interestingly, Abramov et al. [48] reported that A β causes a loss of mitochondrial potential in astrocytes but not in neurons. Since this

effect was prevented by antioxidants and reversed by provision of glutamate and other mitochondrial substrates to complexes I and II, they suggested that the depolarization reflects oxidative damage to metabolic pathways upstream of mitochondrial respiration. However, Paradisi et al. [49] demonstrated that astrocytes can protect neurons from A β neurotoxicity, but when they interact directly with A β , the protection is undermined and the neurotoxicity is enhanced.

It has also been shown that Ntera2 human teratocarcinoma (NT2 rh0+) cells exposed to A β _{25–35} release cytochrome *c*, with subsequent activation of caspases 9 and 3 [50]. Recently, Marques et al. [51] investigated the effect of the A β PP Swedish double mutation (K670M/N671L) on oxidative stress-induced cell death mechanisms in PC12 cells. They observed an increased activity of caspase 3 due to an enhanced activation of both intrinsic and extrinsic apoptotic pathways, including activation of JNK pathway and an attenuation of apoptosis by SP600125, a JNK inhibitor, through protection of mitochondrial dysfunction and reduction of caspase 9 activity. These results support the idea that the massive neurodegeneration at an early age in familial AD patients could be a result of an increased vulnerability of neurons through the activation of different apoptotic pathways as a consequence of elevated levels of oxidative stress.

Yamamori et al. [52] reported that subtoxic concentrations (100–500 nM) of A β _{1–42} can down-regulate the expression of the X-linked inhibitor of apoptosis (XIAP) in human SH-SY5Y neuroblastoma cells and the vulnerability to oxidative stress caused by A β ₄₂ is attenuated by overexpression of XIAP suggesting that XIAP expression in response to subtoxic, more physiological concentrations (100–500 nM) of A β ₄₂ increases the vulnerability to oxidative stress. Song et al. [53] investigated the possibility that overexpression of Bcl-2 may prevent A β -induced cell death through the inhibition of pro-apoptotic activation of p38 mitogen-activated protein kinase (MAPK) and the transcription factor nuclear factor-kappa B (NF- κ B) in nerve growth factor (NGF)-induced differentiated PC12 cells. These results suggest that Bcl-2 overexpression protects against A β -induced cell death of differentiated PC12 and its protective effect may be related to the reduction of A β -induced activation of p38 MAPK and NF- κ B. Tamagno et al. [54] used differentiated SK-N-BE neurons to investigate molecular mechanisms and regulatory pathways underlying apoptotic neuronal cell death elicited by A β _{1–40} and A β _{1–42} peptides as well as the relationship between apoptosis and oxidative stress. They observed that A β peptides, used at concentrations able to induce oxidative stress, elicit a classic type of neuronal apoptosis involving mitochondrial regulatory proteins and pathways (i.e., affecting Bax and Bcl-2 protein levels as well as release of cytochrome *c* in the cytosol), poly-ADP ribose polymerase (PARP) cleavage, and activation of caspase 3. This pattern of neuronal apoptosis is significantly prevented by α -tocopherol and NAC and completely abolished by specific inhibitors of stress-activated protein kinases (SAPK) such as JNKs and p38 MAPK, involved in the early increase of p53 protein levels. These results suggest that oxidative stress-mediated neuronal apoptosis induced by A β operates by eliciting a SAPK-dependent regulation of pro-apoptotic mitochondrial pathways involving both p53 and Bcl2.

The studies presented above clearly show that mitochondrial dysfunction is a key player in AD pathogenesis (Fig. 2).

4. Mitochondrial dysfunction and autophagy in AD

Macroautophagy (thereafter called autophagy) is crucial for neuronal homeostasis, predominantly as housekeeping process to prevent accumulation of protein aggregates and defective organelles (Fig. 3). Neuronal homeostasis depends on balanced, bidirectional trafficking of intracellular constituents between distal neurites and the cell soma. In neurons, autophagosomes and endosomes that fuse in the distal axon must be retrogradely transported to the soma in order to fuse with lysosomes and degrade their contents [55,56]. Thus,

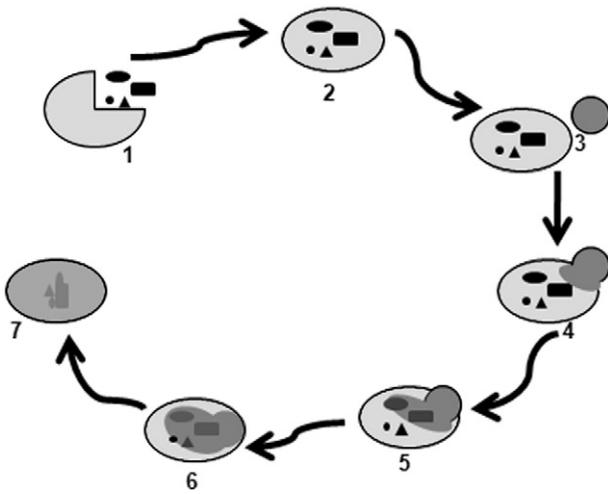


Fig. 3. The autophagic process. The process by which the cell breaks down aggregates of proteins and defective organelles is called (macro)autophagy. These aggregates and defective organelles are engulfed by a double membrane to separate it from the rest of the cell (1); the resulting membrane-enclosed bubble of cytosol and the cells' constituents they contain becomes an autophagosome (2). The autophagosome then fuses with a lysosome that contains several enzymes that can break down the cellular components (3). These enzymes only work in a very acidic environment, so the pH inside lysosomes is much lower than the neutral pH in the rest of the cell. Once the contents of the autophagosome are delivered to the lysosome, its enzymes break down those constituents, which can then be recycled for new use within the cell (4–7).

subtle disruptions of autophagosome formation, maturation, or trafficking would be predicted to have terrible consequences for autophagic flux and neuronal homeostasis. Indeed, evidence is mounting that autophagic pathways are vitally important for the maintenance of neuronal health, particularly in the context of degenerative diseases. Direct evidence for the vital role of autophagy comes from studies of mice which revealed that mice lacking Atg5 [57] or Atg7 [58] showed severe neurodegeneration in the CNS. During recent years, accumulating evidence has been obtained that mitochondria also may be subject to selective degradation (mitophagy). How mitochondria are selectively sequestered into autophagosomes is still largely unknown. Studies in *Saccharomyces cerevisiae* have shown that excess or damaged mitochondria can be selectively eliminated and Uth1 and Aup1 are involved in this process [59–61]. Uth1 is located at the mitochondria outer membrane, whereas Aup1 resides in the intermembrane space of this organelle. Recent data obtained in yeast suggest that mitochondrial fission and fusion processes are important to segregate functional from non-functional regions followed by subsequent autophagic degradation of the non-functional parts [62]. Recently, Kanki and Klionsky [63] found that ATG11, a gene that is essential only for selective autophagy, is also essential for mitophagy. In addition, the authors found that mitophagy is blocked even under severe starvation conditions, if the carbon source makes mitochondria essential for metabolism [63]. These findings suggest that the degradation of mitochondria is a tightly regulated process and that these organelles are largely protected from nonspecific autophagic degradation.

During brain aging, many mitochondria undergo enlargement and structural disorganization, while lysosomes gradually accumulate the nondegradable, polymer lipofuscin. It is believed that this is a result not only of continuous oxidative stress, causing oxidation of mitochondrial constituents and autophagocytosed material, but also of the inherent inability of cells to completely remove oxidatively damaged structures. Although lipofuscin-loaded lysosomes continue to receive newly synthesized lysosomal enzymes, the pigment is nondegradable. Therefore, lipofuscin accumulation may greatly diminish lysosomal degradative capacity by preventing lysosomal enzymes from targeting functional autophagosomes, further limiting

mitochondrial recycling. Based on findings that autophagy is diminished in lipofuscin-loaded cells and that cellular lipofuscin content positively correlates with oxidative stress and mitochondrial damage, Terman and Brunk [56] proposed the mitochondrial-lysosomal axis theory of aging, according to which mitochondrial turnover progressively declines with age, resulting in increased oxidative damage, accumulation of damaged organelles and lipofuscin, decreased ATP production, release of apoptotic factors, and, eventually, cell death (Fig. 4).

Although the exact role of autophagy in AD is not fully defined, recent studies have provided some insights. Nixon et al. [64] identified autophagosomes and other prelysosomal autophagic vacuoles in AD brains particularly within neuritic processes. In dystrophic neurites, the predominant organelles were autophagosomes, multivesicular bodies, multilamellar bodies, and cathepsin-containing autophagolysosomes. Autophagy was evident in the perikarya of affected neurons, particularly in those with neurofibrillary pathology where it was associated with a relative depletion of mitochondria and other organelles [64]. Accordingly, we have recently shown that autophagocytosis of mitochondria is prominent in AD [65,66]. We demonstrated that LA, a sulfur-containing cofactor required for the activity of enzyme complexes central to respiratory metabolism, was localized in pyramidal neurons in cases of AD. Although we observed an age-dependent decrease in LA-binding proteins, we found profound alterations between AD and control cases in terms of LA distribution. We suspect that this could be related to the fragmentation of mitochondrial enzyme complexes. Indeed, electron microscopic analysis showed that, in addition to mitochondria, LA immunoreactivity is also localized in autophagic vacuoles and lipofuscin, whereas control cases present little labeling in the same compartments. Consistent with these observations, light microscopic analysis showed that neurons from cases of AD present granular immunoreactivity, consistent with autophagic vacuoles and increased cytoplasmic staining, when compared with age-matched and young controls [65]. Overall, these observations are in accordance with previous studies from our laboratory and others showing that neurons with increased oxidative damage [5] in AD have a striking and significant increase in mtDNA in neuronal cytoplasm and in vacuoles associated with lipofuscin [18], the proposed site of mitochondrial degradation by autophagy [67]. Consistent with this, we also observed that COX-1, a mitochondrial protein, was increased in the cytosol and associated with mitochondria undergoing phagocytosis [65]. Altogether, these observations support the notion that vulnerable neurons in AD have increased mitochondrial degradation products, suggesting either a greater turnover of mitochondria by autophagy within the cell body and/or a reduction of proteolytic turnover leading to accumulation of products of mitochondria degradation. Interestingly, accumulation of nonprotein components (LA and mtDNA) in the lysosomal pathway is striking while a protein component (COX-1) is absent from these structures. Considering the role of cytosolic mitochondria components in regulating apoptosis and the observation of increased mitochondrial components in the cytosol, possibly resulting from leakage of autophagic components [68], these results merit further investigation. Besides the involvement in mitochondrial turnover, the large autophagic vacuoles found in neurons of AD brains have several other possible implications for AD pathogenesis.

Autophagic vacuoles have been recently shown to contain A β PP as well as the secretase activities required to generate A β and are particularly highly enriched in γ -secretase enzymatic activity and γ -secretase complex components [69,70]. A β ₄₀, A β ₄₂, and β CTF have been detected in purified autophagic vacuoles from livers of yeast artificial chromosome transgenic mice overexpressing A β PP [69]. These observations suggest that accumulated autophagic vacuoles in dystrophic neurites may also contribute to the local production of

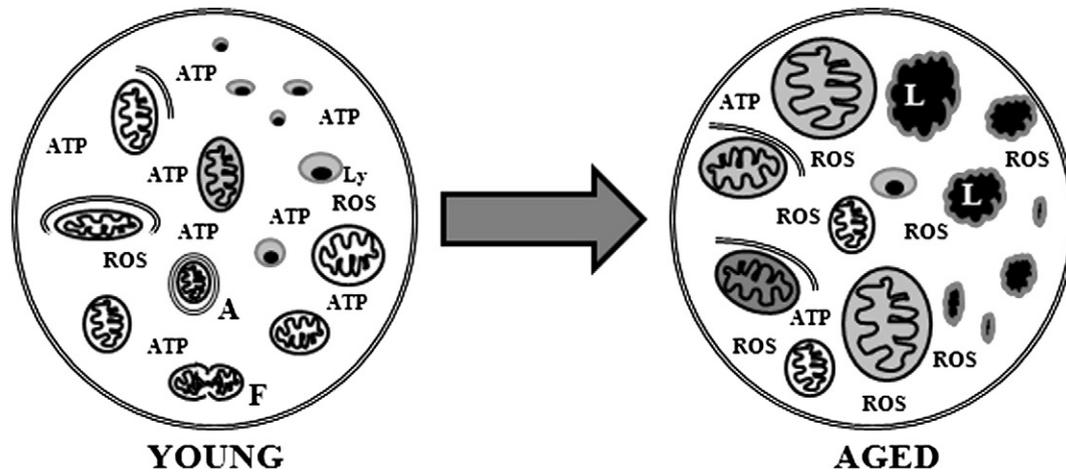


Fig. 4. Schematic representation of the mitochondrial-lysosomal theory of aging. Neuronal cells accumulate damaged mitochondria and lipofuscin inclusions during aging. Oxidative injury to mitochondrial biomolecules may disturb fission of some mitochondria, resulting in their enlargement. Large mitochondria are likely to be poorly autophagocytosed, leading to further damage and enlargement. Lysosomes progressively accumulate lipofuscin pigment. Lipofuscin-containing lysosomes attract large amounts of newly synthesized lysosomal enzymes, which, however, fail to degrade the undegradable pigment. Consequently, it may result in insufficient supply of lysosomal enzymes for autophagocytosis, further lessening mitochondrial recycling. Poorly functioning enlarged mitochondria and lipofuscin-filled lysosomes gradually displace normal organelles, eventually causing decreased ATP production and failing autophagocytosis, resulting in degeneration and death of neuronal cells. A, autophagosome; ATP, adenosine triphosphate; F, mitochondrial fission; L, lipofuscin; Ly, lysosomes; ROS, reactive oxygen species.

A β within plaques and that the generalized increase in autophagy in the neuropil could be a significant source of A β overproduction in AD brain.

Recently, Hung et al. [71] observed that treatment with A β_{25-35} , A β_{42} , or serum starvation induced strong autophagy response in SH-SY5Y overexpressing GFP-LC3. Confocal double-staining image showed that exogenous application of A β_{42} in medium caused the colocalization of A β_{42} with LC3 in neuronal cells. Concomitant treatment of A β with a selective α 7nAChR antagonist, α -bungarotoxin (α -BTX), enhanced A β -induced neurotoxicity in SH-SY5Y cells. On the other hand, nicotine (nAChR agonist) enhanced the autophagic process and also inhibited cell death following A β application. In addition, nicotine but not α -BTX increased primary hippocampal neuronal survival following A β treatment. Furthermore, using Atg7 siRNA to inhibit autophagosome formation in an early step or α 7nAChR siRNA to knock-down α 7nAChR significantly enhanced A β -induced neurotoxicity. Confocal double-staining imaging shows that nicotine treatment in the presence of A β enhanced the colocalization of α 7nAChR with autophagosomes. These results suggest that α 7nAChR may act as a carrier to bind with A β and internalize into cytoplasm and further inhibit A β -induced neurotoxicity via autophagic degradation pathway. These results also suggest that autophagy plays a neuroprotective role against A β -induced neurotoxicity. Defect in autophagic regulation or A β - α 7nAChR transport system may impair the clearance of A β and enhance neuronal death. Ling et al. [72] expressed human A β_{42} and A β_{40} in *Drosophila* neurons. A β_{42} but not A β_{40} causes an extensive accumulation of autophagic vesicles that become increasingly dysfunctional with age. A β_{42} -induced impairment of the degradative function, as well as the structural integrity, of post-lysosomal autophagic vesicles triggers a neurodegenerative cascade that can be enhanced by autophagy activation or partially rescued by autophagy inhibition. Compromise and leakage from post-lysosomal vesicles result in cytosolic acidification, additional damage to membranes and organelles, and erosive destruction of cytoplasm leading to eventual neuron death. The authors indicate that neuronal autophagy initially appears to play a pro-survival role that changes in an age-dependent way to a pro-death role in the context of A β_{42} expression [72]. These observations provide a mechanistic understanding for the differential neurotoxicity of A β_{42} and A β_{40} and reveal an A β_{42} -induced death execution pathway mediated by an age-dependent autophagic-lysosomal injury.

These evidences suggest that autophagy is involved in AD pathophysiology. However, it is not clear yet if autophagy plays a causative role, a protective role or is a consequence of the disease process itself.

5. Can autophagy be considered a therapeutic target in AD?

Autophagy is one of the most rapidly growing fields in biomedical research. The autophagic process is crucial for cells homeostasis, predominantly as housekeeping process to prevent accumulation of protein aggregates and defective organelles. More recently, it has become apparent that levels of autophagy are exquisitely regulated and that autophagy-related processes play central roles in several processes including development, aging, and neurodegeneration.

The role of autophagy as a mediator of cell death has become generally accepted in some fields [73] but alternative interpretations have been proposed [74]. Earlier studies used phosphoinositide 3-kinase inhibitors to inhibit and thus implicate stress-induced autophagy in a pro-death role [75,76]. Other studies used rapamycin to demonstrate an important beneficial role for autophagy in aggregate clearance in neurodegenerative conditions [77]. However, both classes of drugs may have diverse effects on cells that extend beyond autophagy regulation to include modulation of survival/death kinases by 3-methyladenine [75] and regulation of protein synthesis and differentiation through mTOR [78]. Thus, the issue of whether autophagy observed in dying cells reflects a death mechanism, failed adaptation, or epiphenomenon requires additional complementary approaches to establish causality.

Until recently, autophagy has received limited attention in relation to AD despite its importance as a mechanism for removing defective organelles and potentially toxic proteins. Defective autophagy leads to a progressive accumulation within neurons of lipofuscin, defective mitochondria, and cytoplasmic protein aggregates. As a result of lipofuscin accumulation, the proportion of functionally effective structures declines, gradually decreasing adaptability of the biological system. Along these lines, it is possible that lipofuscin deposition hampers autophagic mitochondrial turnover, promoting the accumulation of senescent mitochondria, which are deficient in ATP production but produce increased amounts of ROS. Increased oxidative stress, in turn, further enhances damage to both mitochondria and lysosomes, thus diminishing adaptability, triggering mitochondrial and lysosomal pro-apoptotic pathways, and culminating in

cell death. Furthermore, as a pathogenically important pathway for A β generation and mediator of both cell survival and degenerative phenomena [69–72], autophagy represents a new direction for investigation into the pathogenesis and possible therapy of AD. However, it is essential to clarify the role of autophagy (protective or deleterious) in different stages of AD progression. Indeed, a more complete understanding of (dys)function along the entire spectrum of the autophagic process, from autophagosome formation through clearance and regeneration of new cellular components, is necessary to promote neuronal health and maximize therapeutic potentials.

6. Conclusions

Mitochondria have a wide variety of functions, including ATP production and control of energy efficiency, calcium homeostasis, ROS generation, and apoptotic signaling. Being a major intracellular source of ROS, mitochondria are particularly vulnerable to oxidative stress. Extensive literature exists supporting a causative role of mitochondrial dysfunction and oxidative stress in the pathogenesis of AD. Furthermore, a link between mitochondrial dysfunction/oxidative stress and autophagy has been reported to occur in AD. However, it is not clear yet if autophagy plays a causative role or a protective role or is a consequence of the disease process itself. Understanding the exact role of autophagy in different stages of AD progression may help to design more effective therapeutic strategies. Indeed, a central issue in developing therapies for neurodegenerative diseases involves understanding why and when responses to stress or injury can help prevent neuronal degeneration and death.

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