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Endoplasmic Reticulum Enrollment in Alzheimer's Disease

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Abstract Alzheimer's disease (AD) poses a huge challenge for society and health care worldwide as molecular pathogenesis of the disease is poorly understood and curative treatment does not exist. The mechanisms leading to accelerated neuronal cell death in AD are still largely unknown, but accumulation of misfolded disease-specific proteins has been identified as potentially involved. In the present review, we describe the essential role of endoplasmic reticulum (ER) in AD. Despite the function that mitochondria may play as the central major player in the apoptotic process, accumulating evidence highlights ER as a critical organelle in AD. Stress that impairs ER physiology leads to accumulation of unfolded or misfolded proteins, such as amyloid β (Aβ) peptide, the major component of amyloid plaques. In an attempt to ameliorate the accumulation of unfolded proteins, ER stress triggers a protective cellular mechanism, which includes the unfolded protein response (UPR). However, when activation of the UPR is severe or prolonged enough, the final cellular outcome is pathologic apoptotic cell death. Distinct pathways can be activated in this process, involving stress sensors such as the JNK pathway or ER chaperones such as Bip/GRP94, stress modulators such as Bcl-2 family proteins, or even stress effectors such as caspase-12. Here, we detail the involvement of the ER and associated stress pathways in AD and discuss potential therapeutic strategies targeting ER stress.

Keywords Amyloid β · Caspases · Chaperones · JNK · Tauroursodeoxycholic acid

Abbreviations

AD	Alzheimer's disease
AICD	Amyloid precursor protein intracellular domain
APP	Amyloid precursor protein
ASK1	Apoptosis signal-regulating kinase
ATF	Activating transcription factor
Aβ	Amyloid β
BACE	β-site of APP cleaving enzyme
Bcl-2	B-cell leukemia/lymphoma 2
CHOP	C/EBP homologous protein
eIF2α	Eukaryotic translation initiation factor 2α
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum-associated degradation
GRP	Glucose-regulated protein
GSK-3β	Glycogen synthase kinase-3β
IRE1	Inositol-requiring kinase 1
JNK	c-Jun N-terminal kinase
MAPK	Mitogen-activated protein kinase
MVB	Multivesicular body
NFT	Neurofibrillary tangle
PERK	Protein kinase-like endoplasmic reticulum kinase
PS1	Presenilin-1
RyR	Ryanodine receptor
TRAF2	Receptor-associated factor 2
TUDCA	Tauroursodeoxycholic acid
UPR	Unfolded protein response
UPS	Ubiquitin–proteasome system
XBP1	X-box-binding protein 1

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Introduction

Alzheimer's disease (AD) is a fatal progressive neurodegenerative illness and the most common form of dementia. It is, therefore, a fundamental disorder of cognitive awareness, integrating reasoning, abstraction, language, and memory, one of the defining components of human consciousness [1]. AD is characterized by two main neuropathological hallmarks, including extracellular deposits, known as amyloid or "senile" plaques, and intracellular neurofibrillary tangles (NFTs) [2]. The chief component of intracellular NFTs is tau, a microtubule-associated protein abundant in six different isoforms in the adult brain. Physiologically, tau is a soluble protein present in axons that promotes assembly and stability of microtubules, which are important for vesicle transport. On the contrary, hyperphosphorylated tau, the major form found in AD, is insoluble, lacks affinity for microtubules, and self-associates into paired, helically wound fragments, 10 to 20 nm in diameter, which associate to give insoluble tangles in nerve cell bodies and dendrites [2, 3].

Amyloid plaques are primarily composed of a 4.3-kDa amyloid β (Aβ) peptide. The Aβ peptide is a 39–43 amino acid sequence that forms extraneuronal aggregates with a fibrillar, β-pleated structure. Aβ peptide accumulation can be found both in the brain parenchyma and blood vessels. Aβ is cleaved from amyloid precursor protein (APP) (Fig. 1), a 695–770 amino acid transmembrane protein found in virtually all peripheral and brain cells [4]. Although there is no conclusive evidence of the function of APP, an increasing body of data suggest its involvement in regulating neurite outgrowth, synaptic plasticity, and cell adhesion [5]. APP is normally cleaved within the Aβ domain by α-secretase, originating a soluble N-terminal fragment (sAPPα) and a membrane-bound C-terminal fragment (C83). Alternatively, APP can be cleaved by β-secretase at the N-terminus of the Aβ domain, yielding sAPPβ and C99. The latter membrane-bound fragment then undergoes intramembrane cleavage by γ-secretase at the C-terminus of Aβ, resulting in the release of Aβ into the cell [6]. The secretion of Aβ follows, allowing the peptide to participate in extracellular aggregation and to become incorporated into growing plaques. Importantly, although the classical view is that Aβ is deposited extracellularly, emerging evidence from transgenic mice and human patients indicates that this peptide can also accumulate intraneuronally, which may contribute to disease progression [7].

The β-site of APP cleaving enzyme (BACE1) is responsible for the β-secretase activity, whereas γ-secretase is composed of four essential subunits, including presenilin-1 (PS1) or presenilin-2 (PS2), together with nicastrin, anterior pharynx-defective 1 (APH-1), and presenilin enhancer 2 (PEN-2) [8]. The γ-secretase complex cleaves at multiple sites within the transmembrane domain of APP, generating Aβ peptides ranging in length from 38 to 42 residues [9]. Nearly 90 % of secreted Aβ ends at residue 40, giving Aβ40, whereas Aβ42 accounts for only less than 10 %, and peptides ending at residues 38 are minor components [8, 10]. Importantly, a strict relationship between endoplasmic reticulum (ER) and amyloid secretases has been widely described. Immunohistochemical analyses indicate that PS1 and PS2 are localized to similar intracellular compartments, which include the ER and Golgi complex [11].

The ER fulfills multiple cellular functions (reviewed in [12]). The lumen of the ER is an exceptional compartment, holding the highest concentration of Ca^{2+} within the cell, due to active transport of Ca^{2+} ions by Ca^{2+} ATPases (Fig. 1). In addition, the lumen is an oxidative environment, important for generation of disulfide bonds and proper folding of proteins destined for secretion or for display at the cell surface. Because of its role in protein folding and transport, the ER is also rich in Ca^{2+}-dependent molecular chaperones, such as 78-kDa glucose-regulated protein (GRP78), also known as Bip (GRP78/Bip), 94-kDa glucose-regulated protein (GRP94), and calreticulin, which stabilize protein folding intermediates (reviewed in [13]). Importantly, Ca^{2+} is a vital second messenger associated with the most fundamental molecular pathways within the cell. Thus, its intracellular free levels are tightly regulated by the ER to avoid cell death induced by intracellular Ca^{2+} dysregulation. GRP94 has been extensively linked to cellular Ca^{2+} homoeostasis [14]. One of the major characteristics that GRP94 shares with other ER stress proteins is that its expression is induced through a transcriptional feedback loop [15], when cells are challenged with Ca^{2+} ionophores [16]. In addition, GRP94 binds Ca^{2+} and is one of approximately six luminal proteins that serve as the major Ca^{2+} buffers of the ER [14, 17, 18].

ER stress leads to the activation of several kinases [19] that have profound functional effects on neuronal homeostasis [20, 21]. The ER stress pathway mediated by inositol-requiring kinase 1 (IRE1) activates apoptosis signal-regulating kinase 1 (ASK1), which subsequently can trigger c-Jun N-terminal kinase (JNK) signaling (reviewed in [22]). ASK1-mediated JNK activation has the potential to incite AD pathogenesis [23], through: (i) regulation of APP processing and accumulation of intracellular Aβ [24, 25]; (ii) potentiation of inflammatory responses via activating protein-1 (AP-1) activation [26]; and (iii) phosphorylation of tau protein and aggregation of NFTs [27, 28]. In this review, we will summarize the current knowledge on mechanisms involving or mediated through the ER that may contribute to AD pathogenesis.

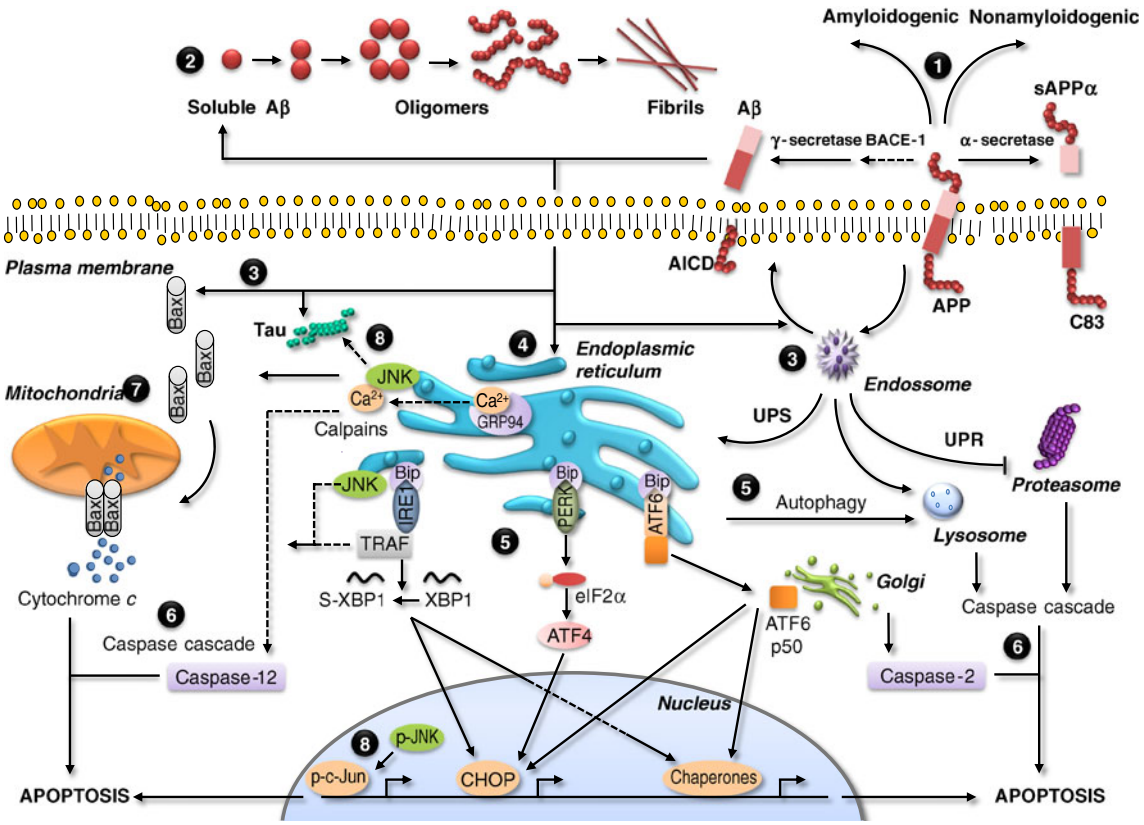

Fig. 1 ER stress in AD. (*1*) APP trafficking, maturation, and processing. After synthesis, APP enters the ER, followed by the Golgi complex, and is transported via the constitutive secretory pathway to the cell membrane (not shown). After reaching the plasma membrane, APP is rapidly internalized and either transported by endocytic and recycling vesicles in a reverse way to the cell surface or degraded in the lysosome. Amyloidogenic processing involves passage through the endocytic organelles, where APP colocalizes with β- and γ-secretases. Nonamyloidogenic processing of APP results from sequential cleavage by membrane-bound α- and γ-secretases. α-Secretase cleaves within the Aβ sequence, thus preventing the generation of intact Aβ peptide. sAPPα ectodomain is secreted to extracellular milieu and C83 is released. Alternatively, amyloidogenic processing starts by β-secretase cleavage at the N-terminus of Aβ, releasing a shorter sAPPβ and C99 fragments (not shown). The C99 fragment is subsequently cleaved within the transmembrane domain by γ-secretase to originate Aβ and AICD. AICD is targeted to the nucleus, signaling transcription activation. (*2*) Aβ assembly states. Soluble Aβ is a hydrophobic peptide that is prone to aggregation. Equilibrium is produced between several extracellular and intracellular Aβ species, including monomeric, oligomeric, and fibrillar forms. All these species originate toxicity through several mechanisms, including microglial infiltration, generation of ROS, and synaptic damage. (*3*) Pathological intracellular Aβ. Aβ, processed either intra- or extracellularly, exerts several toxic effects on organelle and cell function. Intracellular Aβ may contribute to AD pathology by stimulating tau hyperphosphorylation, disrupting proteasome and mitochondria function, and triggering calcium deregulation. (*4*) Deregulation of intracellular Ca^{2+}. Inside the ER, Ca^{2+} is maintained elevated by Ca^{2+}-binding buffering proteins, such as GRP94. Ca^{2+} release from the ER occurs through inositol triphosphate receptors (InsP3Rs or IP3Rs) and ryanodine receptors (RyRs). (*5*) ER protein quality control system. To eliminate misfolded proteins, cells use intracellular, ER-driven protein catabolism mechanisms, namely, the UPS and the autophagy–lysosome pathway or autophagy. ER stress triggers several survival pathways, including UPR that operates to mitigate ER stress via: (i) increasing the expression of chaperone proteins to enhance folding capacity, (ii) inhibiting protein translation via eIF2α, and (iii) promoting the degradation of misfolded proteins through UPS and/or autophagy. Nevertheless, if the response is unable to rescue cells, the ER stress will trigger pathways leading to cell death, including caspase-mediated apoptosis. (*6*) Caspase cascade. Caspase-12 can be activated via IRE1–TRAF2 complex or Ca^{2+}-activated protease calpain during ER stress-induced apoptosis. Aβ-induced cell death requires the activation of caspase-2, which is localized at the Golgi complex and has been described as a target of the JNK pathway that triggers apoptosis through activation of the mitochondrial pathway. (*7*) Mitochondrial apoptotic pathway. Aβ and activated JNK trigger Bax translocation to the mitochondria, formation of pore complex, cytocrome *c* release, and subsequent apoptosis. (*8*) JNK activation. JNK phosporylates tau and triggers its aggregation in NFTs. In addition, JNK activates the proapoptotic transcription factor c-Jun, enhancing the expression of apoptosis proteins

The ER Stress

Disturbances in ER function or its loss of integrity leads to ER stress, which may be caused by accumulation of unfolded proteins or changes in Ca^{2+} homeostasis within the ER [29]. Most neurodegenerative disorders, including AD, are associated with aggregation of misfolded proteins (reviewed in [30]). When viable proteins acquire pathological conformations, physiological functions in the cell are affected, which often culminates in cell death. Pathogenic pathways

involve membrane permeabilization through either a channel mechanism or hydrophobic interaction of prefibrillary oligomers with cellular targets [31].

To eliminate misfolded proteins, cells can activate a large number of intracellular proteases and chaperones, which integrate the ER protein quality control system. The two principal routes of intracellular protein catabolism are the ubiquitin–proteasome system (UPS) and the autophagy–lysosome pathway or autophagy [32]. Both degradation systems incorporate a global process known as ER-associated degradation (ERAD) [33]. In the UPS, ER aberrant proteins are exported to the cytosol and targeted for degradation by covalent attachment of ubiquitin, which is mediated by an enzymatic cascade reaction. The ubiquitin-conjugated proteins are subsequently degraded by a large multisubunit complex, the 26S proteasome [34]. In autophagy, cytoplasmic proteins and/or dysfunctional organelles are sequestered in a double membrane-bound vesicle, termed autophagosome, delivered to the lysosome by fusion and then degraded [35, 36]. Both pathways were described as having a dual role in nervous system homeostasis, including both protection and degeneration [31].

In parallel with ERAD, increased levels of aberrant proteins in the ER activate the unfolded protein response (UPR), a stress response aimed to restore proteostasis in the ER (Fig. 1). Initially cytoprotective, the UPR will trigger a typical apoptotic cascade if the cellular insult is not efficiently removed, representing the last resort of multicellular organisms to dispense dysfunctional cells. The UPR is essential for nonlysosomal degradation and clearance of altered proteins that have the potential to induce cellular damage [37]. It is characterized by the coordinated activation of multiple ER-resident sensors, including double-stranded ribonucleic acid-activated protein kinase-like ER kinase (PERK), IRE1, and activating transcription factor 6 (ATF-6). Once activated, these proteins trigger signaling events, such as increased expression of ER chaperones, inhibition of protein entry into the ER, blockage of mRNA translation, and acceleration of altered protein export from the ER to the cytosol for ubiquitination and proteasome-mediated degradation through the UPS (reviewed in [38]). Normally, the N-termini of these transmembrane ER proteins are held by ER chaperone GRP78/Bip, preventing their activation. However, when misfolded proteins accumulate in the ER, GRP78/Bip is released, allowing activation of these signaling proteins, and launching the UPR [12]. The release of GRP78/Bip permits IRE1 to dimerize, activating both its protein kinase activity through autophosphorylation, and ribonuclease activity. IRE1 dimer binds tumor necrosis factor receptor-associated factor 2 (TRAF2), activating ASK1 and downstream kinases that, in turn, activate p38 mitogen-activated protein kinase (MAPK) and JNK. In addition, through its ribonuclease activity, IRE1 removes a 26-base intron from *X-box-binding protein 1* (*XBP1*) mRNA. The spliced *XBP1* mRNA encodes a potent transcription factor that, following translocation to the nucleus, activates the expression of genes involved in the reestablishment of protein folding or in the degradation of unfolded proteins. The release of GRP78/Bip also results in the activation of PERK, through PERK homodimerization and *trans*-autophosphorylation. Activated PERK then phosphorylates the PERK-eukaryotic translation initiation factor 2α (eIF2α), reducing global mRNA translation, while favoring the translation of selected mRNAs, such as *ATF-4* mRNA. ATF-4 activates the transcription of UPR target genes encoding factors involved in restoring ER homeostasis, via amino acid biosynthesis, antioxidative stress response, apoptosis, and autophagy. In contrast to PERK and IRE1, release of Bip from ATF-6 induces its translocation to the Golgi complex where it is processed by Site-1 (S1P) and Site-2 (S2P) proteases to generate ATF-6α. This fragment migrates to the nucleus, where it activates the transcription of genes mainly involved in ERAD and ER homeostases. Upon severe ER stress, ATF-4, XBP1, and ATF-6 can increase the expression of the proapoptotic transcription factor C/EBP homologous protein (CHOP), which mediates apoptosis by upregulating the proapoptotic BH3-only protein Bim and by suppressing B cell leukemia/lymphoma 2 (Bcl-2) expression. Moreover, CHOP activity is enhanced through phosphorylation by p38 MAPK. In turn, JNK phosphorylation activates Bim, while inhibiting Bcl-2 functions [37].

Importantly, many pathophysiological events of AD associate ER stress with disease progression, including APP subcellular trafficking, maturation, and processing, pathological intracellular Aβ, deregulation of intracellular Ca^{2+}, caspase-12 activation, and JNK activation, among others. As a protective cellular mechanism triggered by increased levels of misfolded proteins, the UPR may be crucial in AD pathogenesis. One arm of this pathway results in the transient shutdown of protein translation, through phosphorylation of eIF2α. Activation of the UPR and/or increased phosphorylated eIF2α levels are seen in patients with neurodegenerative diseases, including AD [39–42], but how this links to neurodegeneration has only recently been uncovered [43]. In fact, it has been shown that accumulation of prion protein during prion replication causes persistent translational repression of global protein synthesis by phosphorylated eIF2α, associated with synaptic failure and neuronal loss in prion-diseased mice. Given the prevalence of protein misfolding and activation of the UPR in several neurodegenerative diseases, these results suggest that manipulation of common pathways such as translational control, rather than disease-specific approaches, may lead to new therapies preventing synaptic failure and neuronal loss across the spectrum of these disorders.

APP Subcellular Trafficking, Maturation, and Processing

APP is a transmembrane protein that is folded and modified in the ER and then transported through the Golgi complex to the outer plasma membrane. In both neuronal and non-neuronal cells, APP is recognized to be transported through the secretory pathway, a continuum transport in separate membrane-enclosed organelles that ultimately reach the cell surface (Fig. 1). Throughout this secretory transport, post-translational modifications of the newly synthesized APP proteins are prone to occur, which may influence APP cleavage and Aβ production. APP processing takes place in various organelles, during its normal secretory pathway, and also at the cell surface. However, it is still not completely understood which cellular compartments process APP to toxic Aβ peptides [44].

Most APP processing occurs after complete maturation of the protein, even though some immature APP may also be cleaved by secretases at a low rate in the ER or the cis-Golgi complex subcellular compartments. Mature APP is processed rapidly, with turnover of ~30–45 min, as it is transported to or from the cell surface via the secretory or endocytic pathways, respectively [45–47]. Interestingly, only small amounts of APP are detected at the cell surface when compared to the total cellular pool [46]. This is the consequence of rapid removal of APP from the cell surface. APP half-life was reported to be shorter than 10 min [48], either by APP proteolytic cleavage or endocytosis. Around 30 % of cell surface APP is processed to sAPP and secreted [45], while the remaining cell surface C-terminal fragments (CTFs) may be cleaved by γ-secretase locally or through the endocytic pathway in endosomes, or further degraded in lysosomes [49]. Both cell surface CTF cleavage product and unprocessed full-length APP are reinternalized through coated pits and vesicles by receptor-mediated endocytosis [47]. If endocytosed, internalized APP half-life is ~30 min [45], with a pool of endosomal APP being delivered to lysosomes for degradation.

APP processing during its trafficking through the different subcellular organelles can originate several APP fragments [50]. However, in addition to Aβ, the physiological or pathophysiological roles of other APP-derived protein fragments cleaved by β- and γ-secretase remain largely unknown. Recent studies elucidated some of the molecular mechanisms for the APP intracellular domain (AICD), the short APP C-terminal region which is generated by γ- and/or ε-secretase cleavage [51]. AICD overexpression has been shown to directly induce apoptosis or to sensitize cells to stress-induced apoptosis [52–56]. Moreover, it has recently been shown that AICD specifically sensitizes cells to ER stress-induced cell death [57].

Altered APP metabolism appears to be a key event in the pathogenesis of AD. Abnormal trafficking, maturation, and processing of APP in ER may promote neuronal degeneration by disrupting the ratio of APP fragments in favor of neurotoxic forms, such as Aβ, rather than of neuroprotective secreted forms, such as sAPPα. Mutations in the human PS1 gene alter proteolytic processing of APP and decrease resistance to apoptosis induced by various cell stresses. In fact, mutations in PS1 influence the UPR under ER stress-favorable conditions [58].

Importantly, recent studies strongly suggest that accumulated intracellular Aβ oligomers can be transmitted neuron to neuron via direct neuritic connections [59]. The mechanism of transmission may involve the lysosomal–endosomal system; however, further studies are needed to confirm this mechanism.

Pathological Intracellular Aβ

There is substantial evidence from transgenic mouse models that intracellular Aβ initiates cellular dysfunction, before it accumulates in extracellular plaques [60]. Moreover, a recent study characterizing intracellular accumulation of Aβ in AD patients concluded that intracellular Aβ was abundantly present but did not correlate with plaque load or NFT formation [61]. In addition, the disease-associated isoform Aβ42 seems to be more prone to intracellular accumulation than Aβ40. Also, intracellular Aβ occurs most frequently in the hippocampus and entorhinal cortex, which are the brain regions affected first in AD [62]. Expression of apolipoprotein E-allele 4 (APOE-ε4), the major genetic risk factor for sporadic AD, also increases intracellular Aβ [63]. Within neurons, Aβ42 seems to localize in multivesicular bodies (MVBs), which are considered late endosomes and are generated from the early endosome system. Immunogold electron microscopy in the brains of AD patients demonstrated Aβ42 localization on the external membrane of MVBs [64]. The accumulation of nonfibrillar Aβ within neuronal MVBs has also been shown in APP/PS1 double transgenic mice model of AD, with Aβ-containing MVBs being frequently observed in the perinuclear region [65]. Furthermore, neurons from APP/PS1 transgenic mice exhibited Aβ-positive granules within the perinuclear region of the cell body, which were largely double-labeled with the lysosomal-associated membrane protein 2 (LAMP2); cathepsin D, a lysosomal hydrolase; and MG160, a Golgi complex marker [65].

Recent studies have demonstrated that Aβ accumulation within MVBs is pathological, leading to disrupted MVB organization through impairment of the UPS [66]. Furthermore, inhibition of the proteasome by Aβ has been demonstrated in animal and cell culture models (Fig. 1) [66, 67]. On the other hand, proteasome inhibition in the 3xTg-AD mice resulted in oligomeric Aβ accumulation within neuronal cell bodies [68, 69]. In addition, proteasome inhibition,

both in vivo and in vitro, resulted in elevated Aβ levels, suggestive that the proteasome degrades Aβ and that Aβ must be within the cytosolic compartment for this degradation to occur [44]. These findings suggest that oligomeric Aβ accumulation within neuronal cell bodies has pathological consequences, including proteasome impairment.

A large body of evidence indicates that the accumulation of intracellular Aβ induces the expression of ER stress markers. Immunohistochemical studies in postmortem brain samples from AD patients indicated the presence of neuronal staining for phosphorylated (activated) UPR kinases, such as PERK and IRE1 [41]. These proteins were found clearly upregulated in hippocampal neurons, particularly in cells containing granulovacuolar degeneration. Interestingly, pPERK-positive neurons also exhibited abundant glycogen synthase kinase-3β (GSK-3β) staining. This is a relevant observation, since it points out that ER stress may trigger the expression of GSK-3β, a well-known tau kinase involved in NFT formation [70].

Nonetheless, the concept that Aβ is an incidental catabolic toxic waste resulting from APP processing has been challenged. Recent studies suggest a physiological role for Aβ as part of a response of the innate immune system, acting as anti-infective antimicrobial peptide (AMP) agent [71]. The exact mechanism is not fully understood; however, Aβ ability to associate with lipid bilayers may be crucial, resulting in either adsorption [72] or permeabilization [73]. Interestingly, oligomerization plays a key role in Aβ membrane targeting, and the pivotal importance of ER in this process (Fig. 1) is well-described. Future studies should further explore the relevance of ER modulation on Aβ anti-infective properties.

Deregulation of Intracellular Ca^{2+}

Disturbances in Ca^{2+} regulation can also induce the UPR and perturb cellular events that control cell fate (Fig. 1). Bcl-2 family proteins represent the strongest link between ER Ca^{2+} regulation and the cell death machinery, as several Bcl-2 proteins reside in the ER membrane and modulate ER Ca^{2+} homeostasis. In fact, Bcl-2 and B-cell leukemia/lymphoma extra long (Bcl-x_L) decrease basal Ca^{2+} concentrations in the ER, whereas Bcl-2-associated X protein (Bax) has an opposite effect [74].

Several studies have demonstrated an association between ER stress and disturbed Ca^{2+} homeostasis in AD pathogenesis [75]. In particular, PS1 mutations associated with familial AD lead to increased susceptibility to stressing agents and cause elevated levels of free Ca^{2+} in PC12 cells and hippocampal neurons from transgenic mice brains [76]. Moreover, mutant PS1-expressing cells show increased Aβ production, altered Ca^{2+} homeostasis [77], and enhanced sensitivity to ER stress-induced apoptosis [29]. Similarly, PS1 mutant transgenic mice display abnormalities in ER Ca^{2+} regulation and increased neuronal vulnerability toward cell death and excitotoxic injury. It has also been demonstrated that mutant PS1 binds to and inhibits the UPR protein IRE1, suppressing activation of the UPR [58].

Interestingly, the brain of early AD patients shows increased expression of ER-resident ryanodine Ca^{2+} channel receptors (RyR) [78], further linking AD to Ca^{2+} dyshomeostasis. Using cellular and animal models of AD, mutant PS1 leads to elevated levels of the type 3 RyR (RyR3) in both PC12 cells and primary neurons [79]. Increased RyR3 isoform was also demonstrated in transgenic mice carrying three mutant AD genes (*APP*, *PS1*, and *tau*) [80, 81] and in transgenic mice expressing triple mutant APP [82]. More recently, the ER stress response factor XBP1 in its active, spliced form was reported as neuroprotective in different AD models by decreasing RyR3 isoform and preventing the accumulation of free Ca^{2+} in the cytosol [83]. Thus, Ca^{2+} dysregulation seems to play a key role as mediator of AD pathogenesis.

Caspase-12 Activation

There is a growing list of mediators linking ER stress to the apoptotic machinery. Caspase-12 was proposed to function as the apical caspase responsible for initiating an apoptotic cascade in response to ER stress and Aβ [84]. Studies to date suggest that the mechanism of ER stress-mediated caspase-12 activation involves the interaction of procaspase-12 with the IRE1–TRAF2 complex [85], but the significance of this interaction remains to be determined. Caspase-12 can also be activated by the calcium-activated protease calpain in settings of ER stress-induced apoptosis [86]. Further evidence linking ER stress and caspase-12 activation came from caspase-12 knockout mice primary cortical neurons, which showed reduced susceptibility towards ER stress. Accordingly, these neurons were resistant to Aβ-induced cell death [84], suggesting that ER stress and activation of caspase-12 may contribute to neuronal death in AD.

JNK Stress Sensor

The activation of the JNK signaling pathway has been identified as a key event in AD-associated apoptosis. The IRE1/TRAF2/ASK1 pathway activates stress kinases (Fig. 1) [19, 22, 37, 87], which have deep functional effects on neuronal homeostasis [20, 21, 88]. ER stress activates ASK1, which subsequently triggers JNK and p38 MAPK signaling. However, evidence suggests that ER stress is not the only inducer of ASK1. In fact, Aβ induces neuronal apoptosis through reactive oxygen species (ROS)-mediated ASK1 activation rather than via ER stress [89]. Nevertheless, ER stress can

activate ROS production via Ca^{2+} mitochondrial signaling. he ASK1-mediated JNK pathway plays a key part in AD pathogenesis [23]. This stress signaling kinase can regulate APP processing [25] and control the phosphorylation state of APP at Thr668 site, which is important for both APP cleavage and degradation in physiological conditions. Inhibition of JNK-mediated phosphorylation of APP causes it to follow the proteasome degradation pathway [90]. JNK may also induce accumulation of intracellular Aβ [24, 25], phosphorylate tau protein, and trigger aggregation of NFTs [27, 28]. Furthermore, JNK mediates the activation of several apoptotic molecules, including caspase-2 [91], p53 [92], and Bcl-2 family members [22, 93], and potentiates inflammatory responses via AP-1 activation [26].

Autophagy

Histopathological analysis of AD brains showed accumulation of activated, phosphorylated JNK (pJNK) in granules within hippocampal pyramidal cells [94]. These granules often colocalize with granulovacuolar degeneration bodies (GVD) [94], which also contain GSK-3β [95], a recognized PERK target kinase. GVD are large cytoplasmic vacuoles [94] that result from autophagic vacuoles usually seen in AD [96]. Interestingly, it has been shown that excessive ER stress can induce autophagic uptake of accumulated material from the overloaded ER [97]. In fact, a recent study suggests that autophagy is the major degradation pathway following UPR activation in neuronal cells, highlighting a connection between UPR activation and autophagic pathology in AD brain [98].

Tau Accumulation

Increased oxidative stress, impaired ER protein-folding function, and deficient proteasome-mediated and autophagic-mediated clearance of damaged proteins are all associated with the accumulation of Aβ and tau proteins in AD [40, 99]. Furthermore, recent research demonstrates that CHOP silencing [100] and UPR activation are intimately connected with the accumulation and aggregation of phosphorylated tau [41, 101].

JNK phosphorylates tau protein at Thr205 and Ser422, which are highly phosphorylated in AD [27] and trigger aggregation of NFTs [28]. Besides JNK, GSK-3β is a crucial kinase believed to have a central role in the hyperphosphorylation of tau present in NFTs [102]. In fact, it has been shown that phosphorylation of tau triggered by ER stress is mediated by GSK-3β, following activation by UPR signaling pathway [103, 104]. Importantly, both activated pJNK and pGSK-3β are present in pretangle accumulations of tau protein [105, 106]. Furthermore, recent data demonstrate that JNK can induce caspase cleavage of tau protein and also that GSK-3β activation is required for tau aggregation [107].

c-Jun Apoptotic Pathway

c-Jun, an immediate–early proapoptotic protein of the JNK pathway, was found to be colocalized with fragmented DNA in neurons [108]. Moreover, enhanced expression of the transcription factors c-Jun and c-Fos, increased levels of c-Jun mRNA, and phosphorylation of c-Jun on its N-terminal transactivation domain have all been observed in neuronal apoptosis [109]. Finally, Aβ itself may induce activation of JNK and c-Jun [110]. Consistently, Aβ-induced cell death is attenuated in cortical neurons from JNK3-null mice, while JNK3 mediates cell death through the activation of c-Jun and enhanced expression of apoptosis antigen-1 ligand (FasL) [110]. More recently, c-Jun has also been shown to be required for Aβ-mediated degradation of antiapoptotic ΔNp63 [111].

Therapeutic Strategies Focused on ER Stress

Targeting neurodegeneration mediated by drugs that modulate ER stress mechanisms is still under evaluation. Screening studies with compounds that impair tunicamycin-induced cell death in a neuronal cell line context highlighted the finding of salubrinal, a compound that prevents dephosphorylation of eIF2α. Consequently, salubrinal increases eIF2α phosphorylation and activation, promoting stronger PERK responses [112]. Salubrinal was described to prevent neuronal cell death triggered by several ER stress inducers [113, 114]. Nevertheless, it was also shown that this compound impairs long-term memory in a mouse model [115], suggesting that its use would not be suitable for chronic therapies. Chemical inhibitors of ASK1 have also been suggested to be cytoprotective in neurodegenerative disorders [116]. Since JNK activation is observed downstream of ASK1, and JNK is known to activate Bid while inhibiting Bcl-2, it would be attractive to investigate whether chemical inhibitors of JNK might also show cytoprotective effects in such context [117].

An additional potential strategy for ameliorating ER stress induced by inclusion bodies is to stimulate autophagy, efficiently clearing insoluble protein aggregates from cells. Chemical screens for enhancers of autophagy have been reported, which have identified compounds that improve clearance of protein inclusions from cultured cells [118, 119]. Among the compounds purported to increase autophagy without signs of cellular toxicity are several drugs already approved by the US Food and Drug Administration. These include antipsychotics (such as fluspirilene, trifluoperazine, and pimozide) and Ca^{2+}-channel modulators (such

as nicardipine, niguldipine, and amiodarone), acting through mechanisms that are distinct from that of rapamycin (a mammalian target of rapamycin (mToR) inhibitor) [118].

A different strategy proposed for removing insoluble protein inclusions is to increase chaperone activity in cells, especially cytosolic heat shock protein 70 (hsp70). It would be interesting to explore the efficacy of chemical chaperones that serve as ligands to stabilize protein structure and promote protein folding, analogous to what has been described for compounds such as SR121463A (1-[4-(Ntertbutyl carbamoyl)-2-methoxybenzene sulphonyl]-5-ethoxy-3-spiro-[4-(2-morpholinoethoxy)cyclohexane] indol-2-one, fumarate) [120] and others [121, 122]. In this regard, the ER-chaperone cyclophilin B [123] and the chemical chaperone phenylbutyric acid [124] have recently been reported as neuroprotective against Aβ-induced toxicity in vitro and in vivo, respectively. Thus, previous studies are encouraging and suggest that targeting the cellular protein quality control system of the ER is an attractive strategy that warrants further exploitation for the treatment of neurodegenerative conditions associated with accumulation of damaged molecules within the cell.

Interestingly, endogenous bile acids, namely, the more hydrophilic molecule tauroursodeoxycholic acid (TUDCA), have been suggested to be strong neuroprotective molecules due to their antiapoptotic properties [125, 126]. Recently, these molecules have been recognized to modulate ER stress-mediated cell death mechanisms [127]. The antiapoptotic effects of TUDCA were firmly established in animal models of AD and cultured neurons incubated with Aβ [128]. Similar results were seen in in vitro models of familial AD that consist of mouse neuroblastoma cells expressing APP with the Swedish mutation or double-mutated for human APP and PS1 [129].

It has been shown that Aβ-induced cell death requires the activation of caspase-2 [91]. Notably, TUDCA prevented caspase-2 activation in neuroblastoma PC12 cells [130]. Caspase-2 has been described as a target of the JNK pathway that triggers apoptosis through activation of the mitochondrial pathway. In this respect, we have shown that TUDCA strongly modulates the mitochondrial pathway, inhibiting Bax translocation triggered by Aβ [131]. In addition, TUDCA abrogated Aβ-induced JNK/caspase-2 signaling [130]. Aβ exposure resulted in activation of the early stress JNK pathway, leading to its nuclear translocation and activation of caspase-2 localized in the Golgi complex. Further investigations are warranted to elucidate the mechanism(s) by which TUDCA interferes with this signaling pathway. Finally, recent data has shown that TUDCA modulates Aβ-induced caspase-12-mediated apoptosis triggered at ER subcellular compartment, independently, however, of ER stress [127]. ER stress markers, including GRP94, ATF-6α, CHOP, and eIF2α, were strongly downregulated by Aβ, independently of protein degradation, and partially restored by TUDCA. Moreover, calpain inhibition prevented caspase-12 activation and ATF-6α downregulation [127].

TUDCA also mitigates the toxic downstream effects of Aβ. In primary rat cortical neurons incubated with fibrillar Aβ42, TUDCA inhibited the levels of apoptosis and caspase-3 activation and abrogated caspase-3 cleavage of tau into toxic species [132]. Thus, by interfering with apoptotic pathways, both at the mitochondrial and transcriptional levels, TUDCA not only increased the survival of neurons but also prevented downstream abnormal conformations of tau. This might have beneficial consequences in slowing cognitive decline. In fact, recent evidence indicates that feeding APP/PS1 double-transgenic mice with diet containing 0.4 % TUDCA for 6 months reduced accumulation of Aβ deposits in the brain, ameliorating learning and memory deficits [133]. TUDCA treatment was shown to decrease Aβ production and intracellular accumulation by reducing lipid metabolism mediators involved in overall amyloidogenic APP processing and Aβ load. Further, TUDCA effectively modulated excitatory synaptic deficits induced by Aβ [134].

Conclusion

In the present review, we have described the central role of ER in AD. Accumulating evidence highlights the key role of ER stress in AD pathogenesis. In fact, it has been suggested that apoptosis can be induced by the ER stress pathway, independently of mitochondria [135]. Moreover, it is also important to recognize that the ER is in a pivotal position to both respond to and cause dysfunction in other subcellular compartments, such as mitochondria, cytoplasm, and nucleus. Thus, it is common to associate ER stress response with multiple processes originating in other organelles, such as ATP depletion, oxidative stress, mitochondrial dysfunction, and lipid accumulation.

The ER bears a central position in AD etiology, inherent to presenilin location at ER membranes. APP trafficking, maturation, and processing, which ultimately lead to plaque formation are very much dependent on the ER. In fact, some studies advocate that plaque formation results from failure of the ER to catalyze the post-translational processing of Aβ [136]. Furthermore, there is an age-dependent decline in vital chaperones required for catalysis of this process in the ER [137]. Corroborating this idea, ER-resident molecular chaperones such as GRP78 and GRP94 are downregulated in the brains of AD patients and in PS1 mutant cells [58].

ER plays also an important role in Ca^{2+} intracellular signaling. As the principal reservoir of Ca^{2+}, the ER is very sensitive to changes in its homeostasis, ultimately leading to caspase-12 activation-mediated apoptosis upon Ca^{2+} deregulation.

Finally, the ER also mediates the sensing, activation, and modulation of pivotal signaling pathways that typically

occur in AD. The very best example is represented by the JNK stress pathway, which is intrinsically connected to both ER and AD. JNK stress sensor pathway is activated through ER-dependent kinases. Activated JNK is involved in a myriad of AD toxic mechanisms. JNK activates specific enzymes such as caspase-2, mediating apoptosis. In addition, JNK is not confined to the regulation of apoptotic cell death processes, but it also regulates autophagy. Moreover, JNK is involved in APP processing and tau accumulation. Ultimately, JNK is involved in gene expression regulation and nuclear signaling mediated by the activation of the c-Jun transcription factor. Understanding the specific cellular mechanisms responsible for the wide involvement of the ER in AD will bring us one step closer toward the development of more effective therapeutic tools for AD.

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References

1. Nussbaum RL, Ellis CE (2003) Alzheimer's disease and Parkinson's disease. N Engl J Med 348(14):1356–1364
2. Spillantini MG, Goedert M (1998) Tau protein pathology in neurodegenerative diseases. Trends Neurosci 21(10):428–433
3. Querfurth HW, LaFerla FM (2010) Alzheimer's disease. N Engl J Med 362(4):329–344
4. Selkoe DJ, Schenk D (2003) Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. Annu Rev Pharmacol Toxicol 43:545–584
5. Mattson MP (2004) Pathways towards and away from Alzheimer's disease. Nature 430(7000):631–639
6. Yamazaki T, Ihara Y (1998) Effects of specific protease inhibitors on amyloid beta-protein 42 secretion. Neurobiol Aging 19(1 Suppl):S77–79
7. Umeda T, Tomiyama T, Sakama N, Tanaka S, Lambert MP, Klein WL, Mori H (2011) Intraneuronal amyloid beta oligomers cause cell death via endoplasmic reticulum stress, endosomal/lysosomal leakage, and mitochondrial dysfunction in vivo. J Neurosci Res 89(7):1031–1042
8. Thinakaran G, Koo EH (2008) Amyloid precursor protein trafficking, processing, and function. J Biol Chem 283(44):29615–29619
9. Selkoe DJ, Wolfe MS (2007) Presenilin: running with scissors in the membrane. Cell 131(2):215–221
10. Rostagno A, Holton JL, Lashley T, Revesz T, Ghiso J (2010) Cerebral amyloidosis: amyloid subunits, mutants and phenotypes. Cell Mol Life Sci 67(4):581–600
11. Kovacs DM, Fausett HJ, Page KJ, Kim TW, Moir RD, Merriam DE, Hollister RD, Hallmark OG, Mancini R, Felsenstein KM, Hyman BT, Tanzi RE, Wasco W (1996) Alzheimer-associated presenilins 1 and 2: neuronal expression in brain and localization to intracellular membranes in mammalian cells. Nat Med 2 (2):224–229
12. Xu C, Bailly-Maitre B, Reed JC (2005) Endoplasmic reticulum stress: cell life and death decisions. J Clin Invest 115(10):2656–2664
13. Orrenius S, Zhivotovsky B, Nicotera P (2003) Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 4 (7):552–565
14. Macer DR, Koch GL (1988) Identification of a set of calcium-binding proteins in reticuloplasm, the luminal content of the endoplasmic reticulum. J Cell Sci 91(Pt 1):61–70
15. Little E, Ramakrishnan M, Roy B, Gazit G, Lee AS (1994) The glucose-regulated proteins (GRP78 and GRP94): functions, gene regulation, and applications. Crit Rev Eukaryot Gene Expr 4 (1):1–18
16. Drummond IA, Lee AS, Resendez E Jr, Steinhardt RA (1987) Depletion of intracellular calcium stores by calcium ionophore A23187 induces the genes for glucose-regulated proteins in hamster fibroblasts. J Biol Chem 262(26):12801–12805
17. Nigam SK, Goldberg AL, Ho S, Rohde MF, Bush KT, Sherman M (1994) A set of endoplasmic reticulum proteins possessing properties of molecular chaperones includes Ca(2+)-binding proteins and members of the thioredoxin superfamily. J Biol Chem 269(3):1744–1749
18. Cala SE, Jones LR (1994) GRP94 resides within cardiac sarcoplasmic reticulum vesicles and is phosphorylated by casein kinase II. J Biol Chem 269(8):5926–5931
19. Urano F, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, Ron D (2000) Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science 287(5453):664–666
20. Bogoyevitch MA, Kobe B (2006) Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. Microbiol Mol Biol Rev 70(4):1061–1095
21. Sekine Y, Takeda K, Ichijo H (2006) The ASK1-MAP kinase signaling in ER stress and neurodegenerative diseases. Curr Mol Med 6(1):87–97
22. Tabas I, Ron D (2011) Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. Nat Cell Biol 13 (3):184–190
23. Okazawa H, Estus S (2002) The JNK/c-Jun cascade and Alzheimer's disease. Am J Alzheimers Dis Other Demen 17(2):79–88
24. Shoji M, Iwakami N, Takeuchi S, Waragai M, Suzuki M, Kanazawa I, Lippa CF, Ono S, Okazawa H (2000) JNK activation is associated with intracellular beta-amyloid accumulation. Brain Res Mol Brain Res 85(1–2):221–233
25. Colombo A, Bastone A, Ploia C, Sclip A, Salmona M, Forloni G, Borsello T (2009) JNK regulates APP cleavage and degradation in a model of Alzheimer's disease. Neurobiol Dis 33(3):518–525
26. Manning AM, Davis RJ (2003) Targeting JNK for therapeutic benefit: from junk to gold? Nat Rev Drug Discov 2(7):554–565
27. Reynolds CH, Utton MA, Gibb GM, Yates A, Anderton BH (1997) Stress-activated protein kinase/c-jun N-terminal kinase phosphorylates tau protein. J Neurochem 68(4):1736–1744
28. Vogel J, Anand VS, Ludwig B, Nawoschik S, Dunlop J, Braithwaite SP (2009) The JNK pathway amplifies and drives subcellular changes in tau phosphorylation. Neuropharmacol 57 (5–6):539–550
29. Lindholm D, Wootz H, Korhonen L (2006) ER stress and neurodegenerative diseases. Cell Death Differ 13(3):385–392
30. Doyle KM, Kennedy D, Gorman AM, Gupta S, Healy SJ, Samali A (2011) Unfolded proteins and endoplasmic reticulum stress in neurodegenerative disorders. J Cell Mol Med 15(10):2025–2039
31. Jellinger KA (2009) Recent advances in our understanding of neurodegeneration. J Neural Transm 116(9):1111–1162
32. Scheper W, Nijholt DA, Hoozemans JJ (2011) The unfolded protein response and proteostasis in Alzheimer disease: preferential activation of autophagy by endoplasmic reticulum stress. Autophagy 7(8):910–911

33. Hoseki J, Ushioda R, Nagata K (2010) Mechanism and components of endoplasmic reticulum-associated degradation. J Biochem 147 (1):19-25
34. Upadhya SC, Hegde AN (2007) Role of the ubiquitin proteasome system in Alzheimer's disease. BMC Biochem 8(Suppl 1):S12
35. Yan L, Vatner DE, Kim SJ, Ge H, Masurekar M, Massover WH, Yang G, Matsui Y, Sadoshima J, Vatner SF (2005) Autophagy in chronically ischemic myocardium. Proc Natl Acad Sci USA 102 (39):13807–13812
36. Hoyer-Hansen M, Jaattela M (2007) Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. Cell Death Differ 14(9):1576–1582
37. Kim I, Xu W, Reed JC (2008) Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov 7(12):1013–1030
38. Schroder M, Kaufman RJ (2005) ER stress and the unfolded protein response. Mutat Res 569(1–2):29–63
39. Hoozemans JJ, van Haastert ES, Eikelenboom P, de Vos RA, Rozemuller JM, Scheper W (2007) Activation of the unfolded protein response in Parkinson's disease. Biochem Biophys Res Commun 354(3):707–711
40. Hoozemans JJ, Veerhuis R, Van Haastert ES, Rozemuller JM, Baas F, Eikelenboom P, Scheper W (2005) The unfolded protein response is activated in Alzheimer's disease. Acta Neuropathol 110(2):165–172
41. Hoozemans JJ, van Haastert ES, Nijholt DA, Rozemuller AJ, Eikelenboom P, Scheper W (2009) The unfolded protein response is activated in pretangle neurons in Alzheimer's disease hippocampus. Am J Pathol 174(4):1241–1251
42. Unterberger U, Hoftberger R, Gelpi E, Flicker H, Budka H, Voigtlander T (2006) Endoplasmic reticulum stress features are prominent in Alzheimer disease but not in prion diseases in vivo. J Neuropathol Exp Neurol 65(4):348–357
43. Moreno JA, Radford H, Peretti D, Steinert JR, Verity N, Martin MG, Halliday M, Morgan J, Dinsdale D, Ortori CA, Barrett DA, Tsaytler P, Bertolotti A, Willis AE, Bushell M, Mallucci GR (2012) Sustained translational repression by eIF2alpha-P mediates prion neurodegeneration. Nature 485(7399):507–511
44. LaFerla FM, Green KN, Oddo S (2007) Intracellular amyloid-beta in Alzheimer's disease. Nat Rev Neurosci 8(7):499–509
45. Koo EH, Squazzo SL, Selkoe DJ, Koo CH (1996) Trafficking of cell-surface amyloid beta-protein precursor. I. Secretion, endocytosis and recycling as detected by labeled monoclonal antibody. J Cell Sci 109(Pt 5):991–998
46. Kuentzel SL, Ali SM, Altman RA, Greenberg BD, Raub TJ (1993) The Alzheimer beta-amyloid protein precursor/protease nexin-II is cleaved by secretase in a trans-Golgi secretory compartment in human neuroglioma cells. Biochem J 295(Pt 2):367–378
47. Yamazaki T, Koo EH, Selkoe DJ (1996) Trafficking of cell-surface amyloid beta-protein precursor. II. Endocytosis, recycling and lysosomal targeting detected by immunolocalization. J Cell Sci 109(Pt 5):999–1008
48. Lai A, Sisodia SS, Trowbridge IS (1995) Characterization of sorting signals in the beta-amyloid precursor protein cytoplasmic domain. J Biol Chem 270(8):3565–3573
49. Kaether C, Schmitt S, Willem M, Haass C (2006) Amyloid precursor protein and Notch intracellular domains are generated after transport of their precursors to the cell surface. Traffic 7 (4):408–415
50. Hartmann T (1999) Intracellular biology of Alzheimer's disease amyloid beta peptide. Eur Arch Psychiatry Clin Neurosci 249 (6):291–298
51. Muller T, Meyer HE, Egensperger R, Marcus K (2008) The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease. Prog Neurobiol 85(4):393–406
52. Kim HS, Kim EM, Lee JP, Park CH, Kim S, Seo JH, Chang KA, Yu E, Jeong SJ, Chong YH, Suh YH (2003) C-terminal fragments of amyloid precursor protein exert neurotoxicity by inducing glycogen synthase kinase-3beta expression. FASEB J 17 (13):1951–1953
53. Kinoshita A, Whelan CM, Berezovska O, Hyman BT (2002) The gamma secretase-generated carboxyl-terminal domain of the amyloid precursor protein induces apoptosis via Tip60 in H4 cells. J Biol Chem 277(32):28530–28536
54. Nakayama K, Ohkawara T, Hiratochi M, Koh CS, Nagase H (2008) The intracellular domain of amyloid precursor protein induces neuron-specific apoptosis. Neurosci Lett 444(2):127–131
55. Ozaki T, Li Y, Kikuchi H, Tomita T, Iwatsubo T, Nakagawara A (2006) The intracellular domain of the amyloid precursor protein (AICD) enhances the p53-mediated apoptosis. Biochem Biophys Res Commun 351(1):57–63
56. Passer B, Pellegrini L, Russo C, Siegel RM, Lenardo MJ, Schettini G, Bachmann M, Tabaton M, D'Adamio L (2000) Generation of an apoptotic intracellular peptide by gamma-secretase cleavage of Alzheimer's amyloid beta protein precursor. J Alzheimers Dis 2 (3–4):289–301
57. Kogel D, Concannon CG, Muller T, Konig H, Bonner C, Poeschel S, Chang S, Egensperger R, Prehn JH (2011) The APP intracellular domain (AICD) potentiates ER stress-induced apoptosis. Neurobiol Aging [Epub ahead of print]
58. Katayama T, Imaizumi K, Sato N, Miyoshi K, Kudo T, Hitomi J, Morihara T, Yoneda T, Gomi F, Mori Y, Nakano Y, Takeda J, Tsuda T, Itoyama Y, Murayama O, Takashima A, St George-Hyslop P, Takeda M, Tohyama M (1999) Presenilin-1 mutations downregulate the signalling pathway of the unfolded-protein response. Nat Cell Biol 1(8):479–485
59. Nath S, Agholme L, Kurudenkandy FR, Granseth B, Marcusson J, Hallbeck M (2012) Spreading of neurodegenerative pathology via neuron-to-neuron transmission of beta-amyloid. J Neurosci 32 (26):8767–8777
60. Hsia AY, Masliah E, McConlogue L, Yu GQ, Tatsuno G, Hu K, Kholodenko D, Malenka RC, Nicoll RA, Mucke L (1999) Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. Proc Natl Acad Sci USA 96(6):3228–3233
61. Wegiel J, Kuchna I, Nowicki K, Frackowiak J, Mazur-Kolecka B, Imaki H, Mehta PD, Silverman WP, Reisberg B, Deleon M, Wisniewski T, Pirttilla T, Frey H, Lehtimaki T, Kivimaki T, Visser FE, Kamphorst W, Potempska A, Bolton D, Currie JR, Miller DL (2007) Intraneuronal Abeta immunoreactivity is not a predictor of brain amyloidosis-beta or neurofibrillary degeneration. Acta Neuropathol 113(4):389–402
62. Gouras GK, Tsai J, Naslund J, Vincent B, Edgar M, Checler F, Greenfield JP, Haroutunian V, Buxbaum JD, Xu H, Greengard P, Relkin NR (2000) Intraneuronal Abeta42 accumulation in human brain. Am J Pathol 156(1):15–20
63. Zerbinatti CV, Wahrle SE, Kim H, Cam JA, Bales K, Paul SM, Holtzman DM, Bu G (2006) Apolipoprotein E and low density lipoprotein receptor-related protein facilitate intraneuronal Abeta42 accumulation in amyloid model mice. J Biol Chem 281(47):36180–36186
64. Takahashi RH, Milner TA, Li F, Nam EE, Edgar MA, Yamaguchi H, Beal MF, Xu H, Greengard P, Gouras GK (2002) Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. Am J Pathol 161(5):1869–1879
65. Langui D, Girardot N, El Hachimi KH, Allinquant B, Blanchard V, Pradier L, Duyckaerts C (2004) Subcellular topography of

neuronal Abeta peptide in APPxPS1 transgenic mice. Am J Pathol 165(5):1465–1477
66. Almeida CG, Takahashi RH, Gouras GK (2006) Beta-amyloid accumulation impairs multivesicular body sorting by inhibiting the ubiquitin–proteasome system. J Neurosci 26(16):4277–4288
67. Oh S, Hong HS, Hwang E, Sim HJ, Lee W, Shin SJ, Mook-Jung I (2005) Amyloid peptide attenuates the proteasome activity in neuronal cells. Mech Ageing Dev 126(12):1292–1299
68. Oddo S, Caccamo A, Tran L, Lambert MP, Glabe CG, Klein WL, LaFerla FM (2006) Temporal profile of amyloid-beta (Abeta) oligomerization in an in vivo model of Alzheimer disease. A link between Abeta and tau pathology. J Biol Chem 281(3):1599–1604
69. Tseng BP, Green KN, Chan JL, Blurton-Jones M, LaFerla FM (2008) Abeta inhibits the proteasome and enhances amyloid and tau accumulation. Neurobiol Aging 29(11):1607–1618
70. Resende R, Ferreiro E, Pereira C, Oliveira CR (2008) ER stress is involved in Abeta-induced GSK-3beta activation and tau phosphorylation. J Neurosci Res 86(9):2091–2099
71. Schluesener HJ, Su Y, Ebrahimi A, Pouladsaz D (2012) Antimicrobial peptides in the brain: neuropeptides and amyloid. Front Biosci (Schol Ed) 4:1375–1380
72. Lukiw WJ, Cui JG, Yuan LY, Bhattacharjee PS, Corkern M, Clement C, Kammerman EM, Ball MJ, Zhao Y, Sullivan PM, Hill JM (2010) Acyclovir or Abeta42 peptides attenuate HSV-1-induced miRNA-146a levels in human primary brain cells. NeuroReport 21(14):922–927
73. Soscia SJ, Kirby JE, Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, Burton MA, Goldstein LE, Duong S, Tanzi RE, Moir RD (2010) The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. PLoS One 5(3):e9505
74. Foyouzi-Youssefi R, Arnaudeau S, Borner C, Kelley WL, Tschopp J, Lew DP, Demaurex N, Krause KH (2000) Bcl-2 decreases the free Ca2+ concentration within the endoplasmic reticulum. Proc Natl Acad Sci USA 97(11):5723–5728
75. LaFerla FM (2002) Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. Nat Rev Neurosci 3(11):862–872
76. Guo Q, Fu W, Sopher BL, Miller MW, Ware CB, Martin GM, Mattson MP (1999) Increased vulnerability of hippocampal neurons to excitotoxic necrosis in presenilin-1 mutant knock-in mice. Nat Med 5(1):101–106
77. Levitan D, Lee J, Song L, Manning R, Wong G, Parker E, Zhang L (2001) PS1 N- and C-terminal fragments form a complex that functions in APP processing and Notch signaling. Proc Natl Acad Sci USA 98(21):12186–12190
78. Kelliher M, Fastbom J, Cowburn RF, Bonkale W, Ohm TG, Ravid R, Sorrentino V, O'Neill C (1999) Alterations in the ryanodine receptor calcium release channel correlate with Alzheimer's disease neurofibrillary and beta-amyloid pathologies. Neurosci 92 (2):499–513
79. Chan SL, Mayne M, Holden CP, Geiger JD, Mattson MP (2000) Presenilin-1 mutations increase levels of ryanodine receptors and calcium release in PC12 cells and cortical neurons. J Biol Chem 275(24):18195–18200
80. Smith IF, Hitt B, Green KN, Oddo S, LaFerla FM (2005) Enhanced caffeine-induced Ca2+ release in the 3xTg-AD mouse model of Alzheimer's disease. J Neurochem 94(6):1711–1718
81. Stutzmann GE, Smith I, Caccamo A, Oddo S, Laferla FM, Parker I (2006) Enhanced ryanodine receptor recruitment contributes to Ca2+ disruptions in young, adult, and aged Alzheimer's disease mice. J Neurosci 26(19):5180–5189
82. Supnet C, Grant J, Kong H, Westaway D, Mayne M (2006) Amyloid-beta-(1-42) increases ryanodine receptor-3 expression and function in neurons of TgCRND8 mice. J Biol Chem 281 (50):38440–38447
83. Casas-Tinto S, Zhang Y, Sanchez-Garcia J, Gomez-Velazquez M, Rincon-Limas DE, Fernandez-Funez P (2011) The ER stress factor XBP1s prevents amyloid-beta neurotoxicity. Hum Mol Genet 20(11):2144–2160
84. Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, Yuan J (2000) Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-beta. Nature 403 (6765):98–103
85. Yoneda T, Imaizumi K, Oono K, Yui D, Gomi F, Katayama T, Tohyama M (2001) Activation of caspase-12, an endoplastic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. J Biol Chem 276(17):13935–13940
86. Nakagawa T, Yuan J (2000) Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis. J Cell Biol 150(4):887–894
87. Salminen A, Kauppinen A, Suuronen T, Kaarniranta K, Ojala J (2009) ER stress in Alzheimer's disease: a novel neuronal trigger for inflammation and Alzheimer's pathology. J Neuroinflammation 6:41
88. Dhanasekaran DN, Reddy EP (2008) JNK signaling in apoptosis. Oncogene 27(48):6245–6251
89. Kadowaki H, Nishitoh H, Urano F, Sadamitsu C, Matsuzawa A, Takeda K, Masutani H, Yodoi J, Urano Y, Nagano T, Ichijo H (2005) Amyloid beta induces neuronal cell death through ROS-mediated ASK1 activation. Cell Death Differ 12(1):19–24
90. Colombo A, Repici M, Pesaresi M, Santambrogio S, Forloni G, Borsello T (2007) The TAT-JNK inhibitor peptide interferes with beta amyloid protein stability. Cell Death Differ 14(10):1845–1848
91. Troy CM, Rabacchi SA, Friedman WJ, Frappier TF, Brown K, Shelanski ML (2000) Caspase-2 mediates neuronal cell death induced by beta-amyloid. J Neurosci 20(4):1386–1392
92. She QB, Ma WY, Dong Z (2002) Role of MAP kinases in UVB-induced phosphorylation of p53 at serine 20. Oncogene 21 (10):1580–1589
93. Sorenson CM (2004) Bcl-2 family members and disease. Biochim Biophys Acta 1644(2–3):169–177
94. Lagalwar S, Berry RW, Binder LI (2007) Relation of hippocampal phospho-SAPK/JNK granules in Alzheimer's disease and tauopathies to granulovacuolar degeneration bodies. Acta Neuropathol 113(1):63–73
95. Leroy K, Boutajangout A, Authelet M, Woodgett JR, Anderton BH, Brion JP (2002) The active form of glycogen synthase kinase-3beta is associated with granulovacuolar degeneration in neurons in Alzheimer's disease. Acta Neuropathol 103(2):91–99
96. Nixon RA (2007) Autophagy, amyloidogenesis and Alzheimer disease. J Cell Sci 120(Pt 23):4081–4091
97. Yorimitsu T, Klionsky DJ (2007) Eating the endoplasmic reticulum: quality control by autophagy. Trends Cell Biol 17(6):279–285
98. Nijholt DA, de Graaf TR, van Haastert ES, Oliveira AO, Berkers CR, Zwart R, Ovaa H, Baas F, Hoozemans JJ, Scheper W (2011) Endoplasmic reticulum stress activates autophagy but not the proteasome in neuronal cells: implications for Alzheimer's disease. Cell Death Differ 18(6):1071–1081
99. Lopez Salon M, Morelli L, Castano EM, Soto EF, Pasquini JM (2000) Defective ubiquitination of cerebral proteins in Alzheimer's disease. J Neurosci Res 62(2):302–310
100. Prasanthi JR, Larson T, Schommer J, Ghribi O (2011) Silencing GADD153/CHOP gene expression protects against Alzheimer's disease-like pathology induced by 27-hydroxycholesterol in rabbit hippocampus. PLoS One 6(10):e26420
101. Nijholt DA, van Haastert ES, Rozemuller AJ, Scheper W, Hoozemans JJ (2012) The unfolded protein response is associated with early tau pathology in the hippocampus of tauopathies. J Pathol 226(5):693–702

102. Jope RS, Johnson GV (2004) The glamour and gloom of glycogen synthase kinase-3. Trends Biochem Sci 29(2):95–102
103. Kim AJ, Shi Y, Austin RC, Werstuck GH (2005) Valproate protects cells from ER stress-induced lipid accumulation and apoptosis by inhibiting glycogen synthase kinase-3. J Cell Sci 118(Pt 1):89–99
104. Song L, De Sarno P, Jope RS (2002) Central role of glycogen synthase kinase-3beta in endoplasmic reticulum stress-induced caspase-3 activation. J Biol Chem 277(47):44701–44708
105. Zhu X, Raina AK, Rottkamp CA, Aliev G, Perry G, Boux H, Smith MA (2001) Activation and redistribution of c-jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimer's disease. J Neurochem 76(2):435–441
106. Ishizawa T, Sahara N, Ishiguro K, Kersh J, McGowan E, Lewis J, Hutton M, Dickson DW, Yen SH (2003) Co-localization of glycogen synthase kinase-3 with neurofibrillary tangles and granulovacuolar degeneration in transgenic mice. Am J Pathol 163 (3):1057–1067
107. Sahara N, Murayama M, Lee B, Park JM, Lagalwar S, Binder LI, Takashima A (2008) Active c-jun N-terminal kinase induces caspase cleavage of tau and additional phosphorylation by GSK-3beta is required for tau aggregation. Eur J Neurosci 27 (11):2897–2906
108. Anderson AJ, Su JH, Cotman CW (1996) DNA damage and apoptosis in Alzheimer's disease: colocalization with c-Jun immunoreactivity, relationship to brain area, and effect of postmortem delay. J Neurosci 16(5):1710–1719
109. Sastry PS, Rao KS (2000) Apoptosis and the nervous system. J Neurochem 74(1):1–20
110. Morishima Y, Gotoh Y, Zieg J, Barrett T, Takano H, Flavell R, Davis RJ, Shirasaki Y, Greenberg ME (2001) Beta-amyloid induces neuronal apoptosis via a mechanism that involves the c-Jun N-terminal kinase pathway and the induction of Fas ligand. J Neurosci 21(19):7551–7560
111. Fonseca MB, Nunes AF, Rodrigues CM (2012) c-Jun regulates the stability of anti-apoptotic deltaNp63 in amyloid-beta-induced apoptosis. J Alzheimers Dis 28(3):685-694
112. Boyce M, Bryant KF, Jousse C, Long K, Harding HP, Scheuner D, Kaufman RJ, Ma D, Coen DM, Ron D, Yuan J (2005) A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. Science 307(5711):935–939
113. Smith WW, Jiang H, Pei Z, Tanaka Y, Morita H, Sawa A, Dawson VL, Dawson TM, Ross CA (2005) Endoplasmic reticulum stress and mitochondrial cell death pathways mediate A53T mutant alpha-synuclein-induced toxicity. Hum Mol Genet 14(24):3801–3811
114. Reijonen S, Putkonen N, Norremolle A, Lindholm D, Korhonen L (2008) Inhibition of endoplasmic reticulum stress counteracts neuronal cell death and protein aggregation caused by N-terminal mutant huntingtin proteins. Exp Cell Res 314 (5):950–960
115. Costa-Mattioli M, Gobert D, Stern E, Gamache K, Colina R, Cuello C, Sossin W, Kaufman R, Pelletier J, Rosenblum K, Krnjevic K, Lacaille JC, Nader K, Sonenberg N (2007) eIF2alpha phosphorylation bidirectionally regulates the switch from short- to long-term synaptic plasticity and memory. Cell 129(1):195–206
116. Kawaguchi M, Terai T, Utata R, Kato M, Tsuganezawa K, Tanaka A, Kojima H, Okabe T, Nagano T (2008) Development of a novel fluorescent probe for fluorescence correlation spectroscopic detection of kinase inhibitors. Bioorg Med Chem Lett 18(13):3752–3755
117. Salh B (2007) c-Jun N-terminal kinases as potential therapeutic targets. Expert Opin Ther Targets 11(10):1339–1353
118. Zhang L, Yu J, Pan H, Hu P, Hao Y, Cai W, Zhu H, Yu AD, Xie X, Ma D, Yuan J (2007) Small molecule regulators of autophagy identified by an image-based high-throughput screen. Proc Natl Acad Sci USA 104(48):19023–19028
119. Sarkar S, Perlstein EO, Imarisio S, Pineau S, Cordenier A, Maglathlin RL, Webster JA, Lewis TA, O'Kane CJ, Schreiber SL, Rubinsztein DC (2007) Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. Nat Chem Biol 3(6):331–338
120. Serradeil-Le Gal C, Lacour C, Valette G, Garcia G, Foulon L, Galindo G, Bankir L, Pouzet B, Guillon G, Barberis C, Chicot D, Jard S, Vilain P, Garcia C, Marty E, Raufaste D, Brossard G, Nisato D, Maffrand JP, Le Fur G (1996) Characterization of SR 121463A, a highly potent and selective, orally active vasopressin V2 receptor antagonist. J Clin Invest 98(12):2729–2738
121. Burrows JA, Willis LK, Perlmutter DH (2000) Chemical chaperones mediate increased secretion of mutant alpha 1-antitrypsin (alpha 1-AT) Z: a potential pharmacological strategy for prevention of liver injury and emphysema in alpha 1-AT deficiency. Proc Natl Acad Sci USA 97(4):1796–1801
122. Tamarappoo BK, Verkman AS (1998) Defective aquaporin-2 trafficking in nephrogenic diabetes insipidus and correction by chemical chaperones. J Clin Invest 101(10):2257–2267
123. Oh Y, Kim EY, Kim Y, Jin J, Jin BK, Jahng GH, Jung MH, Park C, Kang I, Ha J, Choe W (2011) Neuroprotective effects of overexpressed cyclophilin B against Abeta-induced neurotoxicity in PC12 cells. Free Radic Biol Med 51 (4):905-920
124. Wiley JC, Pettan-Brewer C, Ladiges WC (2011) Phenylbutyric acid reduces amyloid plaques and rescues cognitive behavior in AD transgenic mice. Aging Cell 10 (3):418-428
125. Rodrigues CM, Stieers CL, Keene CD, Ma X, Kren BT, Low WC, Steer CJ (2000) Tauroursodeoxycholic acid partially prevents apoptosis induced by 3-nitropropionic acid: evidence for a mitochondrial pathway independent of the permeability transition. J Neurochem 75(6):2368–2379
126. Sola S, Castro RE, Laires PA, Steer CJ, Rodrigues CM (2003) Tauroursodeoxycholic acid prevents amyloid-beta peptide-induced neuronal death via a phosphatidylinositol 3-kinase-dependent signaling pathway. Mol Med 9(9–12):226–234
127. Viana RJ, Steer CJ, Rodrigues CM (2011) Amyloid-beta peptide-induced secretion of endoplasmic reticulum chaperone glycoprotein GRP94. J Alzheimers Dis 27 (1):61-73
128. Ramalho RM, Ribeiro PS, Sola S, Castro RE, Steer CJ, Rodrigues CM (2004) Inhibition of the E2F-1/p53/Bax pathway by tauroursodeoxycholic acid in amyloid beta-peptide-induced apoptosis of PC12 cells. J Neurochem 90(3):567–575
129. Ramalho RM, Borralho PM, Castro RE, Sola S, Steer CJ, Rodrigues CM (2006) Tauroursodeoxycholic acid modulates p53-mediated apoptosis in Alzheimer's disease mutant neuroblastoma cells. J Neurochem 98(5):1610–1618
130. Viana RJ, Ramalho RM, Nunes AF, Steer CJ, Rodrigues CM (2010) Modulation of amyloid-beta peptide-induced toxicity through inhibition of JNK nuclear localization and caspase-2 activation. J Alzheimers Dis 22(2):557–68
131. Viana RJ, Nunes AF, Castro RE, Ramalho RM, Meyerson J, Fossati S, Ghiso J, Rostagno A, Rodrigues CM (2009) Tauroursodeoxycholic acid prevents E22Q Alzheimer's Abeta toxicity in human cerebral endothelial cells. Cell Mol Life Sci 66(6):1094–1104
132. Ramalho RM, Viana RJ, Castro RE, Steer CJ, Low WC, Rodrigues CM (2008) Apoptosis in transgenic mice expressing the P301L mutated form of human tau. Mol Med 14(5–6):309–317
133. Nunes AF, Amaral JD, Lo AC, Fonseca MB, Viana RJ, Callaerts-Vegh Z, D'Hooge R, Rodrigues CM (2012)

TUDCA, a bile acid, attenuates amyloid precursor protein processing and amyloid-beta deposition in APP/PS1 mice. Mol Neurobiol 45(3):440-54

134. Ramalho RM, Nunes AF, Dias RB, Amaral JD, Lo AC, D'Hooge R, Sebastiao AM, Rodrigues CM (2012) Tauroursodeoxycholic acid suppresses amyloid beta-induced synaptic toxicity in vitro and in APP/PS1 mice. Neurobiol Aging [Epub ahead of print]
135. Lamarca V, Scorrano L (2009) When separation means death: killing through the mitochondria, but starting from the endoplasmic reticulum. EMBO J 28(12):1681–1683
136. Erickson RR, Dunning LM, Olson DA, Cohen SJ, Davis AT, Wood WG, Kratzke RA, Holtzman JL (2005) In cerebrospinal fluid ER chaperones ERp57 and calreticulin bind beta-amyloid. Biochem Biophys Res Commun 332(1):50–57
137. Erickson RR, Dunning LM, Holtzman JL (2006) The effect of aging on the chaperone concentrations in the hepatic, endoplasmic reticulum of male rats: the possible role of protein misfolding due to the loss of chaperones in the decline in physiological function seen with age. J Gerontol A Biol Sci Med Sci 61 (5):435–443