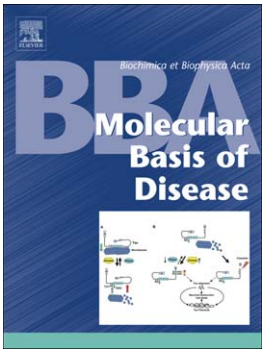

The role of endoplasmic reticulum in amyloid precursor protein processing and trafficking: Implications for Alzheimers disease

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The role of endoplasmic reticulum in amyloid precursor protein processing and trafficking: Implication´s for Alzheimer´s disease

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Abstract

The endoplasmic reticulum (ER) is the principal organelle responsible for the proper folding/processing of nascent proteins and perturbed ER function leads to a state known as ER stress. Mammalian cells try to overcome ER stress through a set of protein signalling pathways and transcription factors termed the unfolded protein response (UPR). However, under unresolvable ER stress conditions, the UPR is hyperactivated inducing cell dysfunction and death. The accumulation of misfolded proteins in the brain of Alzheimer's disease (AD) patients suggests that alterations in ER homeostasis might be implicated in the neurodegenerative events that characterize this disorder. This review discusses the involvement of ER stress in the pathogenesis of AD, focusing the processing and trafficking of the AD-related amyloid precursor protein (APP) during disease development. The potential role of ER as a therapeutic target in AD will also be debated.

Keywords: Alzheimer's disease; amyloid precursor protein; endoplasmic reticulum; secretory pathway; therapeutic targets; trafficking

Abbreviations

Endoplasmic reticulum, ER; unfolded protein response, UPR; Alzheimer disease, AD; amyloid precursor protein, APP; chaperone glucose-regulated protein 78, GRP78/BiP; protein kinase RNA (PKR)-like ER kinase, PERK; activating transcription factor 6, ATF6; inositol-requiring enzyme-1alpha, IRE1α; Xbox binding protein-1, XBP1; ER-associated degradation, ERAD; DnaJ homolog 4, ERdj4; protein kinase inhibitor of 58kDa , $p58^{IPK}$;ER degradation-enhancing α-mannosidase-like protein EDEM; ribosome-associated membrane protein 4, RAMP-4; protein disulfide isomerase P5, PDI-P5; c-Jun NH(2)-terminal kinase, JNK; apoptosis signal-regulating kinase 1, ASK1; nuclear factor kappa-light-chain-enhancer of activated B cells, NF-kB; B-cell lymphoma 2, Bcl-2; p53 upregulated modulator of apoptosis, PUMA; B-cell lymphoma 2 interacting mediator of cell death, BIM; amyloid beta, Aβ; presenilin, PSN; central nervous system, CNS; guanosine triphosphate, GTP; death receptor 6, DR6; beta-secretase, BACE; trans-Golgi network, TGN; protein disulfide isomerase, PDI; 4-phenylbutyric acid, PBA; tauroursodeoxycholic acid, TUDCA; trimethylamine oxide, TMAO; Salubrinal, (3-phenyl-N-[2,2,2-trichloro-1-[[(8-quinolinylamino) thioxomethyl] amino]ethyl]-2-propen amide); BiP inducer X, BIX; dibenzoylmethane, DBM; derivatives, and N-acetyl cysteine (NAC)

1. Endoplasmic reticulum stress

The endoplasmic reticulum (ER) was first described in 1945 as an extensive network of interconnected membrane tubules that spread throughout the cytosol [1]. A large number of studies showed that the ER can be divided into three domains according to its structure and function: 1) the nuclear envelope, which controls the flow of information between the cytoplasm and the nucleus, 2) the sheet-like cisternae, also denominated rough ER due to the high content in ribosomes, and 3) the polygonal array of tubules, also called smooth ER [1, 2]. This highly dynamic and multifunctional organelle is implicated in protein quality control along the secretory pathway being responsible for protein folding, assembly and post-translational modifications (e.g. glycosylation, disulfide bond formation), among other functions.

1.1. The unfolded protein response and its role in cell survival and apoptosis

Perturbations of ER homeostasis, triggered by several factors including ER Ca^{2+} depletion, oxidative stress and mutated proteins that traffic through the secretory pathway can be responsible for the accumulation of misfolded/malfolded proteins in its lumen leading to ER stress. To re-establish homeostasis, the ER activates the unfolded protein response (UPR) [3, 4], which prevents the aggregation and facilitates the folding of damaged proteins, decreases translation to prevent overload of ER lumen with newly synthesized proteins, increases ER biogenesis and volume through the stimulation of lipid synthesis and activates protein degradation via the ER-associated protein degradation (ERAD) pathway [5-7].

In mammals, the mechanisms implicated in the ER stress response are poorly understood. The most accepted hypothesis defends that the ER chaperone glucose-regulated protein 78 (GRP78/BiP) binds the ER stress sensors protein kinase RNA (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme-1alpha (IRE1α) [4]. Under ER stress, GRP78/BiP dissociates from these sensors and promotes their activation, inducing phosphorylation and oligomerization of PERK and IRE1α and the translocation of ATF6 to the Golgi where it is cleaved [4]. Once activated, the ER stress sensors increase several transcription factors and control the expression of chaperones and other modulators of protein quality control within the secretory pathway [4]. After the onset of ER stress, the activation of the three branches of the UPR occurs in a time-dependent manner

(Fig. 1) [8, 9].

Upon activation, PERK phosphorylates eIF2α on the serine 51 of its α subunit, which leads to the inhibition of delivery of the initiator methionyl-tRNA to the ribosome, resulting in general inhibition of protein translation [9, 10]. Paradoxically, eIF2α phosphorylation also promotes the translation of selective mRNAs that contain the internal entry ribosomal site (IRES), leading to the translation of genes associated with UPR, namely the transcription factor gene 4 (ATF4) [10, 11]. This transcription factor is responsible for the upregulation of genes associated with redox homeostasis, energy metabolism and protein folding [3, 4].

The activation of IRE1α triggers the selective degradation of mRNAs encoding for proteins with abnormal folding, induces the unconventional splicing of the mRNA encoding the transcription factor Xbox binding protein-1 (XBP1), which shifts the coding reading frame and leads to the expression of a more stable and active transcription factor, XBP1s. XBP1s is responsible for the regulation of a subset of UPR target genes related with protein folding, ER/Golgi biogenesis and ERAD, namely endoplasmic reticulum DnaJ homolog 4 (ERdj4), protein kinase inhibitor of 58kDa ($p58^{IPK)}$, ER degradation-enhancing α-mannosidase-like protein (EDEM), ribosome-associated membrane protein 4 (RAMP-4), protein disulfide isomerase P5 (PDI-P5) and HEDJ [12]. In addition, IRE1α interacts with several adaptor proteins, such as c-Jun NH(2)-terminal kinase (JNK), apoptosis signal-regulating kinase 1 (ASK1), the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), and can thus trigger autophagy, apoptosis and/or an inflammatory response [4].

ATF6 is a membrane-spanning protein that after dissociation from GRP78/BiP translocates to the Golgi where it is activated through proteolytic processing. In the nucleus, active ATF6 induces the expression of genes associated with protein quality control mechanisms [13]. This transcription factor can act synergistically with XBP1s [3].

Although the UPR is activated in order to restore organelle and cellular homeostasis, prolonged UPR activation can trigger apoptosis (Fig. 2) [4, 14]. Besides the pro-survival effect discussed above, the IRE1α/XBP1 pathway has an important role in apoptosis. Indeed, the phosphorylation of IRE1α by the c-Jun-N-terminal inhibitory kinase leads to the recruitment of the tumor necrosis factor receptor associated-factor-2 (TRAF2). The complex IRE1-TRAF2 activates caspase-12 and a

downstream caspase cascade is activated [15]. The complex IRE1-TRAF2 also activates a Jun NH(2)-terminal kinase (JNK)-mediated apoptotic pathway [15]. IRE1α also promotes the selective degradation of mRNAs encoding proteins of the secretory pathway, a process called regulated IRE1-dependent decay (RIDD) [16]. Initially, RIDD was proposed as an adaptive mechanism to reduce the overload of proteins in the secretory pathway but recent studies suggested that RIDD-mediated degradation of mRNA that encode ER chaperones such as GRP78/BiP acts as a pro-apoptotic stimulus. Sustained activation of PERK can induce the major pro-apoptotic transcription factor C/EBP homologous protein CHOP/GADD153 [17], which upregulates the ER oxidase 1α (ERO1α), leading to excessive generation of oxidant species and depletion of the antioxidant glutathione [18, 19] and also to the release of Ca^{2+} from the ER through the IP3R [20, 21]. In addition, the ER stress-induced transcription factor CHOP/GADD153 can downregulate the anti-apoptotic Bcl-2 and upregulate the pro-apoptotic proteins Bax and Bak [21].

The ATF6 signalling pathway seems to predominantly play a pro-survival role and only few evidence links ATF6 and cell death. Recently, this transcription factor has been associated with the downregulation of the anti-apoptotic Mcl-1 in myoblasts during differentiation, however this effect has not been reported in an ER stress context [22, 23].

1.2. ER calcium homeostasis and crosstalk with mitochondria

In the ER, the homeostasis of Ca^{2+} is maintained due to the concerted action of Ca^{2+} pumps that actively uptake Ca^{2+}, Ca^{2+}-binding proteins that allow the storage of Ca^{2+} in the ER lumen and Ca^{2+} channels that release Ca^{2+} into the cytosol in response to several stimuli [4, 19, 24]. The Ca^{2+} channels associated with the ER receptors for IP3 (IP3R) and ryanodine (RyR) are present at the mitochondria-associated membrane (MAM), which is responsible for the communication between the ER and the mitochondria [4, 25]. MAM allows the formation of Ca^{2+} microdomains that ensure an efficient transfer of Ca^{2+} between ER and mitochondria, essential for energetic metabolism and cell survival [4]. ER-to-mitochondria Ca^{2+} transfer can be regulated by the ER chaperone Sigma-1 receptor (Sigma-1R) [4, 26]. Impairment of Ca^{2+} coupling between the ER and mitochondria can lead to apoptosis [27] and is tightly modulated by members of the Bcl-2 family [28]. The induction of apoptosis

depends of the amount of anti- and pro-apoptotic proteins at the ER membrane [4, 28]. The anti-apoptotic proteins can regulate ER Ca^{2+} levels through the interaction with the IP3R, however the underlying mechanisms remain unknown [28]. It was shown that the deletion of pro-apoptotic proteins Bax and Bak promotes the interaction between IP3R and anti-apoptotic Bcl-2 family members, consequently decreasing Ca^{2+} released from ER stores [28-30].

It was hypothesized that the presence of Bax at the ER surface under ER stress conditions is responsible for the release of Ca^{2+} from ER by blocking the interaction between Bcl-xL and the IP3R subsequently leading to the activation of the ER resident caspase-12 [29, 30]. In rodents, caspase-12 is an ER membrane pro-apoptotic cysteine protease activated under ER stress conditions. This caspase was silenced in humans by evolutionary mutations and it is believed that caspase-4 plays a similar role to caspase-12 in rodents [31]. The activation of caspase-12 occurs through cleavage of its prodomain and cleaved caspase-12 activates the apoptosis' effector caspase-3 via caspase-9, independently of cytochrome c and Apaf-1 [31-33].

In summary, the UPR is a stress response strategy that regulates protein folding/misfolding at the ER and controls cell fate. This protein quality control mechanism operating at the ER is able to trigger adaptive and protective pathways, however, chronic UPR activation in neurodegenerative diseases such as Alzheimer's disease (AD) can have a negative impact on cell survival since it affects the folding and trafficking of proteins through the secretory pathway [34, 35].

2. Alzheimer's disease and ER stress

AD is the most common form of dementia in the elderly, which affects more than 30 million people worldwide and 10 million in Europe [36] and it is believed that AD pathogenesis is related to the synergistic interaction between ageing and a multitude of cellular, biochemical and genetic factors. Mutations in presenilin 1 (PSN1), presenilin 2 (PSN2) and amyloid precursor protein (APP) lead to early-onset familial AD, prior to age 65. Specific polymorphisms in apolipoprotein E are associated with risk to develop the most frequent, late-onset form of AD, occurring after 65 years [37].

The clinical symptoms of AD are characterized by a progressive cognitive deterioration together with impairments in behavior, language, and visuospatial skills,

culminating in the premature death of the individual. These traits are accompanied by neuropathological features observed in postmortem AD brains, including a selective neuronal and synaptic loss in cortical and subcortical regions, deposition of extracellular senile plaques, mainly composed of amyloid β (Aβ) peptide, presence of intracellular neurofibrillary tangles (NFT) containing hyperphosphorylated tau protein, and cerebral amyloid angiopathy [38].

A growing number of studies suggest that Aβ interferes with ER functioning leading to ER stress in early stages of AD progression [39-41]. In fact, it was shown that exogenous Aβ induces ER stress in primary neuronal cultures and activates mitochondria- and ER-mediated cell death pathways [42-44]. In PC12 cells and knock-in mice expressing mutant PS1 it was shown that the upregulation of protein levels of CHOP/GADD153 was followed by the downregulation of the anti-apoptotic protein Bcl-2. Concomitantly, the authors observed that mutant PS1 sensitizes cells to the deleterious effects of ER stress culminating in cell death, this effect being attenuated by anti-sense-mediated suppression of CHOP/GADD153 production [45]. In the hippocampus of the triple transgenic mouse model of AD (3xTg-AD) increased levels of CHOP/GADD153 precede the increase in BACE and Aβ levels [46].

In AD brains, the ER stress marker GRP78/BiP is positively correlated with Braak staging [40]. Moreover, analysis of AD postmortem brain revealed an alteration in protein levels of several ER stress markers, namely GRP78/BiP, protein disulfide isomerase (PDI), PERK, eIF2α posphorylation and IRE1α [4, 47-54].

3. The Amyloid Precursor Protein

3.1. APP structure and function

APP is a type I transmembrane protein formed by a large extracellular N-terminal domain, a transmembrane domain and a short C-terminal cytoplasmic domain composed of 59 amino acid residues [55]. Although APP can be detected in all tissues, it is highly expressed in the brain. In mammals, the APP gene is located in the long arm of the chromosome 21. There are 8 isoforms of APP, generated by alternative splicing, that range from 365 to 770 amino acids residues and the most common isoforms contain 695, 751 and 770 amino acids. The 695 isoform is highly expressed in the central nervous system (CNS) and the others are ubiquitously

expressed in all tissues.

Since the identification of APP as the precursor protein of Aβ a large number of physiological functions have been proposed to APP. In 1993, Nishimoto *et al* suggested that the cytoplasmic domain of APP is a receptor coupled to a guanosine triphosphate (GTP)-binding protein [56]. Studies involving APP overexpression demonstrated that it positively modulates cell survival and growth [57, 58]. Saitoh *et al* showed that the normal growth of fibroblasts that harbor an antisense construct of APP can be restored with the administration of the secreted form of APP (sAPP) [59]. Accordingly, sAPP was demonstrated to reduce neuronal apoptosis in models of traumatic brain injury [60]. However, in 2009 Oh *et al.* observed that in both transgenic mouse models with the familial AD *Swedish* mutation and mice carrying *wild type* APP the overexpression of APP increases the percentage of medium size neurons and decreases the percentage of small-sized neurons suggesting that APP induces the hypertrophy of neurons [61] that was believed to be a compensatory mechanism or a reaction to neuronal injury. Similarly, Leyssen *et al.* showed that APP promotes neurite arborization using a drosophila model of brain injury [62]. APP also plays an important role in the maintenance and formation of synapses, neuronal survival and neuritic outgrowth [63-66]. In the embryonic cortex of rodents the knock down of APP was found to be responsible for abnormal synaptic activity and neuronal migration [67-69]. Moreover, in 2009 it was proposed that APP ectodomain can act as a ligand for the death receptor 6 (DR6) and, according to the studies of Nikolaev *et al.*, growth factor deprivation triggers APP cleavage by beta-secretase 1 (BACE1) resulting in the release of APP ectodomain, which binds to DRP6 and activates caspases -3 and -6 inducing apoptosis [70]. APP may also act as an adhesion molecule or adhesion receptor of growth cones [71]. Indeed, this protein is highly expressed in axons and has the ability to bind to extracellular matrix components such as collagen I, glypican, heparin and co-localize with integrins and laminin [69, 72-75]. Finally, it was proposed that the C-terminal domain of APP acts as a transcriptional regulator of its own intracellular sorting [76]. Despite the numerous studies reporting the relevance of APP in neuronal cells, others show that APP knockout causes only subtle phenotypic alterations [77-79]. These discrepancies need to be carefully examined to uncover the importance of APP, particularly in the CNS.

Aβ itself plays an important role in synaptic regulation. In addition, it was

suggested that neuronal activity regulates, at least in part, the production of Aβ, supporting the idea that this peptide has a physiological function [80, 81]. Accordingly, some studies showed that small increases of Aβ levels, within the physiological range, enhance the release of synaptic vesicles and increase neuronal activity [76, 82].

3.2. Role of ER stress in APP processing and trafficking

Despite the large number of studies devoted to clarify the mechanisms involved in the processing of APP, it is presently unclear if different APP isoforms are processed by the same metabolic pathway and if they similarly trigger the amyloidogenic pathway. It is known that APP protein has three main proteolytic cleavage sites that lead to APP processing through distinct catabolic pathways. More than 90% of APP is cleaved by the non-amyloidogenic pathway involving the zinc metalloproteinase α-secretase, releasing a secreted form of APP (sAPPα). The C-terminal fragment (C-88) that is retained in the membrane is subsequently cleaved by a γ-secretase generating a short fragment called p3 and a cytoplasmic fragment identified as the APP intracellular domain (AICD). In the non-amyloidogenic pathway, α-secretase cleaves APP within the Aβ sequence precluding Aβ formation. APP can also be processed through the less common amyloidogenic pathway, which generates Aβ through the sequential cleavage by β-secretase (BACE) and γ-secretase (Fig. 3) [36, 76, 83]. Presently, the exact subcellular localization of APP processing remains unclear. Lammich *et al* [84] suggested that the non-amyloidogenic processing of APP occurs predominantly in the plasma membrane. It is believed that the majority of APP is processed after maturation and transport to the cell surface via the secretory pathway but it can also occur upon internalization of plasma membrane-located APP through the endocytic pathway [85].

It has been reported that the transport of APP from ER to the plasma membrane occurs differently in non-polarized and polarized cells, such as neurons. Soma, dendrites and axons play different functions and have different sets of proteins and lipids that regulate protein trafficking [85]. In neurons, the delivery of proteins to the proper destination is ensured by microtubules, motor proteins (dinein and kinesin) and sorting signals. During its trafficking from the ER to the plasma membrane, APP undergoes several post-translational modifications. After protein synthesis in

membrane-bound polysomes, APP is N-glycosylated in the ER and then transported to the Golgi apparatus where it is O- and N-glycosylated, phosphorylated and sulphonated at tyrosine motifs [86]. In vitro studies have shown that only 10% of APP goes to the plasma membrane and that the majority of APP protein remains in the Golgi and trans-Golgi network (TGN). APP is transported along the axon in post TGN vesicles or in elongated tubular structures using kinesin 1 as a motor protein. According to Kaether *et al.* APP tubules move unidirectionally using fast axonal transport that can reach velocities of 10 μm/s [87, 88]. The fate of APP-loaded vesicles transported along axons remains unknown. It was suggested that a small fraction of axonal APP undergoes transcytosis for transport to dendrites but no signaling sorting transport has been identified yet [85, 89]. Studies performed in non-polarized cells suggest that APP inserted in the plasma membrane can be preferentially cleaved via the non-amyloidogenic pathway and, due to the presence of a "YENPTY" internalization motif near the C-terminus, APP could be internalized via endocytosis reaching the endosome [90]. After endocytosis, APP fragments can return to the cell surface, can be degraded in the lysosome or can be transported in a retromer-dependent manner to the TGN. Recent studies suggest that the APP retromer transported from the early endosome to TGN is relevant for the amyloidogenic processing of APP. Using HEK293 cells expressing APP695, Choy *et al.* [91] showed that depletion of the late endosomal sorting complexes required for transport of ESCRT components, which re-route APP from the endocytic pathway to the TGN, as well as the depletion of the adaptor protein AP-4 that impedes direct TGN-to-endosome trafficking of APP, lead to the amyloidogenic processing of APP in TGN, enhancing Aβ1-40 formation. These results are in contradiction with the study performed by Small *et al.* [92] showing that the depletion of VPS35, which stimulates the retromer-mediated recycling of APP to the TGN, increases the amyloidogenic processing of APP in TGN. More studies are thus required to elucidate the mechanisms implicated in the trafficking of APP in neurons.

In the last few years several studies demonstrated that α- and β-secretases are present in the ER allowing APP processing [93, 94]. Recently, it was shown that PSN1, PSN2, and γ-secretase activity, are located predominantly in a specialized subcompartment of the ER that is physically and biochemically connected to mitochondria, called MAM, which is an intracellular lipid raft-like structure

intimately involved in cholesterol and phospholipid lipid metabolism, in Ca^{2+} homeostasis, and in mitochondrial function and dynamics, and that is increased significantly in AD [95]. Taking into account numerous evidence showing that the amyloidogenic APP processing occurs predominately in lipid-raft domains, [96] it can be hypothesized that the ER-mitochondria interface is a subcellular site of enhanced Aβ generation. Shin *et al* reported that in COS7 cells transfected with APP695, α- and β-secretases compete with each other for the intracellular APP cleavage and that this competition modulates the production of Aβ in the ER [94]. In vitro studies further showed that APP695 accumulates in this organelle upon ER stress while APP751 accumulation occurs preferentially in the Golgi [34] suggesting that sorting and processing of neuronal and non-neuronal APP is mediated by different mechanisms under stress conditions. Moreover, in HEK293 cells transfected with APP, it was observed that APP interacts with GRP78/BiP preventing its translocation to distal compartments, which leads to its sorting into COPI vesicles involved in retrograde transport and accumulation in the ER [97]. The co-precipitation of APP with GRP78/BiP shows that the majority of APP is immature, suggesting that the retrograde transport could be part of the UPR protein quality control system. However, Katayama *et al* observed a decrease in GRP78/BiP levels in samples of grey matter of familial AD patients suggesting that the loss or dysregulation of UPR can initiate neuronal degeneration through Aβ generation as a consequence of amyloidogenic APP processing upon GRP78/BiP downregulation [98]. We can thus hypothesize that Aβ accumulation is not only a cause of ER stress-induced chronic UPR but also a consequence of ER disruption.

Despite the different isoforms of APP and experimental models used, the above-mentioned studies suggest that the retention of APP in the ER leads to an alteration of its processing and trafficking and decreases secretion of Aβ from the ER to the cytosol. It is known that the accumulation of proteins in ER triggers ER stress that, if prolonged, can lead to apoptosis. Recently, it was described that ER stress occurring in HEK293 cells overexpressing APP695 increases the levels of APP mRNA but decreases its protein levels, which results from the cleavage of APP at the α-site and also from the phosphorylation at the tyrosine 687 residue that promotes the γ-secretase-mediated cleavage and the generation of the AICD fragment [99]. This fragment is able to increase the susceptibility to apoptosis via ER stress [99, 100].

However, another study performed in PC12 cells showed that APP*wt* increases the resistance to ER stress-mediated apoptosis and mutant APPsw abolishes this resistance [101]. Similar effects were observed when ER stress was induced with brefeldin A, an inhibitor of the transport between the ER and the Golgi. The authors also showed that the increased resistance to apoptosis under ER stress conditions was not related with changes in the expression of GRP78/BiP or CHOP/GADD153 suggesting that APP acts downstream, or independently, of ER-to-nucleus signaling [101].

4. The ER as a therapeutic target for AD

It was recently suggested that the modulation of the ER-UPR could be a suitable therapeutic strategy to avoid neuronal degeneration in several human diseases, including AD. Indeed, manipulating ER-associated quality control mechanisms through the stimulation of protective and/or adaptive responses or the inhibition of the apoptotic pathways associated with the UPR can be beneficial to treat or prevent AD. The use of chemical chaperones such as 4-phenylbutyric acid (PBA), tauroursodeoxycholic acid (TUDCA) or trimethylamine oxide (TMAO) may be advantageous since these compounds improve ER folding capacity and stabilize protein conformation [102]. In an AD mouse model, PBA was shown to enhance ER function, to prevent Aβ accumulation and to avoid the loss of dendritic spines and memory [103]. TUDCA, a taurine-conjugated derivative from ursodeoxycholic acid, was found to ameliorate microglia and astrocytic activation and to prevent Aβ production in the cortex and hippocampus of APP/PS1 mice through the regulation of APP processing [67]. In this mouse model, TUDCA rescued neurons and improved memory [104]. Although less used, the chemical chaperone TMAO has been shown to be more efficient than PBA and TUDCA to promote protein folding, to reduce accumulation of aggregates and to prevent apoptosis [105] and thus can be considered a promising therapeutic strategy for AD.

Ca^{2+} is an important player in neurodegeneration and many chaperones do not work properly when Ca^{2+} homeostasis is impaired. Targeting ER Ca^{2+} may allow the restoration of ER homeostasis and increase protein folding. The RyR antagonist dantrolene, licensed for the treatment of spasticity, inhibits ER Ca^{2+} release and the activation of PERK, eIF2a and CHOP/GADD153 [102]. In the 3xTg-AD mouse model, dantrolene reduced memory deficits and the burden of neuritic plaques in the

hippocampus, and in primary cultures of cortical neurons this compound partially prevented neuronal death induced by Aβ [106, 107]. Furthermore, the selective inhibitor of the IP3R xestospongin C can also be considered an effective strategy for the treatment of AD. This inhibitor was described to reduce Ca^{2+} levels in SHSY5Y cells transfected with the PS1Δ9 mutation to values similar to those observed in PS1wt cells and in primary cultures of cortical neurons treated with Aβ [107, 108].

Compounds that interact directly with UPR mediators can also be valuable therapeutic agents. Salubrinal (3-phenyl-N-[2,2,2-trichloro-1-[[(8-quinolinylamino) thioxomethyl] amino]ethyl]-2-propen amide) was reported to inhibit the IRE1α/ASK1/JNK signaling pathway and also the GADD34-phosphatase complex that dephosphorylates eIF2a. In PC12 cells it was shown that salubrinal is a protective agent against ER stress. Moreover, studies performed in primary cortical or hippocampal cultures and in the SK-N-SH human neuroblatoma cell line treated with salubrinal observed an increase in peIF2α and an attenuation of ER stress-mediated apoptosis [102, 109-111]. A recent screening has identified a selective inducer of GRP78/BiP [112] named BiP inducer X (BIX) (1-(3,4-dihydroxy-phenyl)-2-thiocyanato-ethanone) that, through the induction of the ER stress-mediated element, is able to up-regulate GRP78/BiP in an ATF6-dependent manner, but IRE1- and PERK-independent manner [102]. BIX also reduces the protein levels of CHOP/GADD153 and cell death in mouse retina [113].

Several studies showed that antioxidant compounds such as edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, dibenzoylmethane (DBM) derivatives, and N-acetyl cysteine (NAC) have beneficial effects under ER stress conditions. Edaravone is a free radical scavenger that provided neuroprotection in several models of disease. It was shown in SH-SY5Y cells that edavorene prevents Aβ production through stimulation of the non-amyloidogenic APP processing [114]. In primary cultures of glia, edavorene attenuated ER stress induced by tunicamycin and reduced cell death through the inhibition of CHOP/GADD153 and XBP1 [115]. Edavorene is also described as an inhibitor of PERK, eIF2a, ATF4 and caspase-12 [116-118]. NAC is another scavenger of oxidant species that presents beneficial effects during ER stress-mediated apoptosis, as suggested by the study of Park et al showing that NAC reverts the downregulation of phospho-PERK, CHOP/GADD153, ATF4, GADD34, GRP78/BiP, and spliced XBP1 in EBV-transformed B cells by cross-linking of CD70

[119].

5. Conclusions

ER has a pivotal role in many cellular processes, such as Ca^{2+} buffering, protein synthesis and transport and apoptotic signalling, which are known to be disrupted in AD. Moreover, growing evidence supports the involvement of ER stress in AD pathogenesis. A potential problem in understanding the pathophysiology of AD might arise from the difficulties in determining the subcellular localization of specific biochemical players under pathological conditions and how protein modifications affect their trafficking and sub-cellular localization. Concerning APP, it seems clear that ER plays a fundamental role in its trafficking and processing. APP is folded in this organelle and undergoes several posttranslational modifications. Therefore, we believe that chronic ER stress, which occurs throughout disease progression, interferes with trafficking of proteins within the secretory pathway, including APP (Fig. 3), leading to the intracellular production of Aβ that, in turn, activates several synaptotoxic and neurotoxic pathways culminating in apoptotic cell death. Accordingly, the modulation of the ER-UPR could be a suitable therapeutic strategy to delay or stop neuronal degeneration in AD. Indeed, several ER stress modulators have emerged in the last years (Fig. 4). However, the UPR can induce both cell survival and death, depending of the duration and intensity of the stimuli. The transient induction of adaptive and protective ER stress-mediated UPR may mitigate neuronal dysfunction delaying the signs of AD and avoid chronic ER stress, which once triggered activates UPR-associated apoptotic pathways that potentiate disease progression. Therefore, selection of compounds that act in the multiple branches of UPR could represent a good strategy to prevent the abnormal processing of APP as well as the deleterious downstream events that characterize AD pathology.

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Figures legends

Figure 1 – The unfolded protein response (UPR). Perturbation of endoplasmic reticulum (ER) homeostasis triggers adaptive signaling cascades associated to the ER stress sensors Xbox binding protein-1 (XBP1), protein kinase RNA (PKR)-like ER kinase (PERK) and inositol-requiring enzyme-1alpha (IRE1α). These ER sensors are inactivated through the interaction with the 78 kDa glucose-regulated protein (GRP78/BIP). However, the accumulation of incorrectly folded proteins in the ER lumen detaches GRP78/BIP from these transmembrane proteins, which become activated. Active PERK phosphorylates the eukaryotic initiation factor-2alpha (eIF2α) at serine 51 reducing protein synthesis and, consequently, protein overload in the ER. eIF2α also activates the transcription factor ATF4, which upregulates UPR target genes encoding factors involved in amino-acid biosynthesis, the antioxidant stress response. The activation of IRE1α leads to non-canonical XBP1 splicing. This spliced form of XBP1 (sXBP1), alone or synergistically with activating transcription factor 6 (ATF6), activates the transcription of UPR target genes. Activated ATF6 migrates to the nucleus to stimulate the expression of genes containing the ER stress response element (ERSE) and the UPR elements.

Figure 2 - Endoplasmic reticulum (ER) stress-mediated apoptosis. Unresolved ER stress induces apoptotic cell death through different mechanisms. The release of Ca^{2+} from ER causes the activation of resident caspase-12 that initiates a caspase cascade. Activating transcription factor 4 (ATF4) induces apoptosis through activation of the transcription factor CHOP/GADD153, which in turn upregulates pro-apoptotic Bcl2 family members such as Bax and Bak and downregulates anti-apoptotic members namely Bcl-xL)). The pro-apoptotic members of the Bcl2 family may trigger apoptosis in a mitochondria-dependent pathway that involves translocation of Bax and Bak from the ER to the mitochondria and also activation of caspases through the release of cytochrome c (Cyt C) from mitochondria. The phosphorylation of c-Jun N-terminal kinase (JNK) that occurs upon interaction of IRE1a and tumor necrosis factor (TNF) receptor-associated factor 2 (TRAF2) also activates the transcription factor CHOP/GADD153.

Figure 3 - Amyloid precursor protein (APP) processing and trafficking in the secretory pathway. After synthesis, APP is folded and undergoes several posttranslational modifications in the ER and Golgi and is transported to the plasma membrane where it can be cleavage by α-secretase. This cleavage occurs within the Aβ peptide sequence, which avoids the formation of Aβ. APP can also be cleavage through the sequential cleavage of β- and γ- secretases to release Aβ. Non-processed APP located at the plasma membrane is rapidly internalized and trafficked via the endocytic pathway to the trans-Golgi network (TGN) and to the ER in a retromer-dependent manner. Once in the ER, APP can generate Aβ due to the action of ER secretases.

Figure 4 - The ER stress-induced UPR signaling pathways as therapeutic targets. The chemical chaperones PBA, TUDCA and TMAO improve protein folding and down regulate the levels of GRP78/BiP. PBA is also described as an inhibitor of peIF2α CHOP/GADD153, XBP1, caspase-12 and ATF6. TUDCA downregulates peIF2α, CHOP/GADD153 and XBP1. The inhibitor of GADD34-phosphatase complex salubrinal downregulates CHOP/GADD153, caspase 12 and ATF6 and upregulates the ER components eIF2α, GRP78/BiP, ATF4 and XBP1. Dantrolene and xestospongin C interfere with Ca^{2+} signaling at the ER and regulates the activity of PERK and IRE1α.

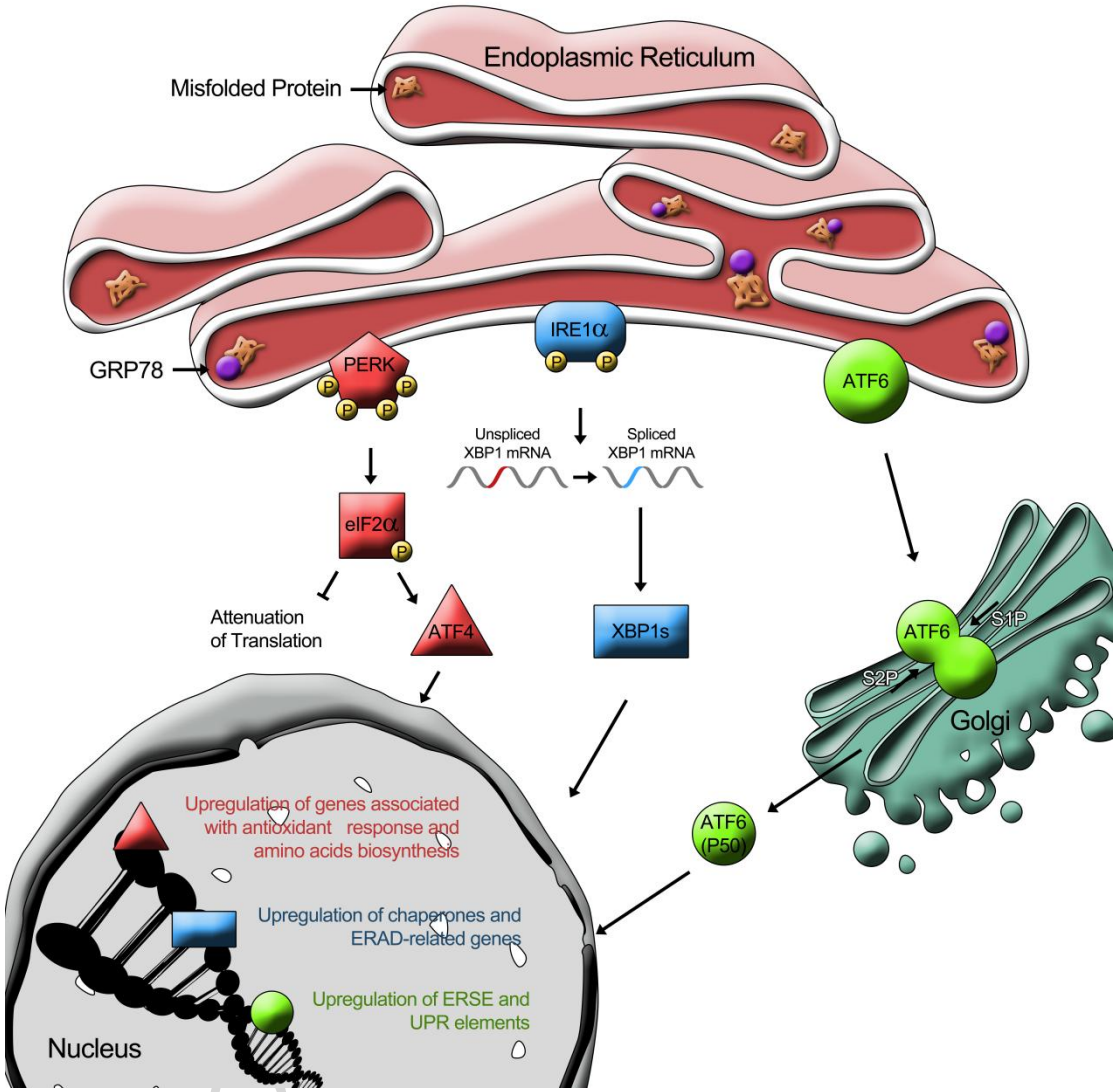

Figure 1

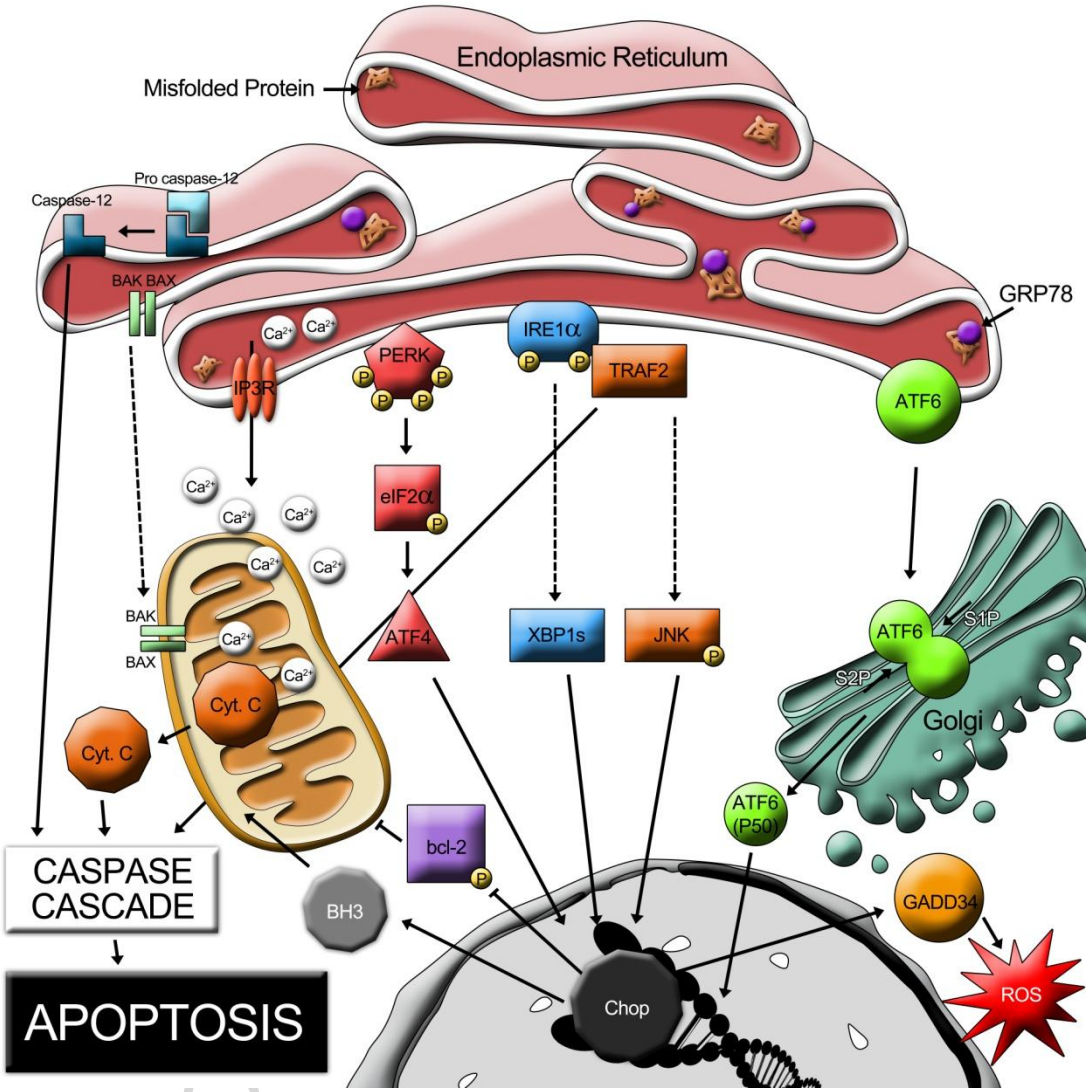

Figure 2

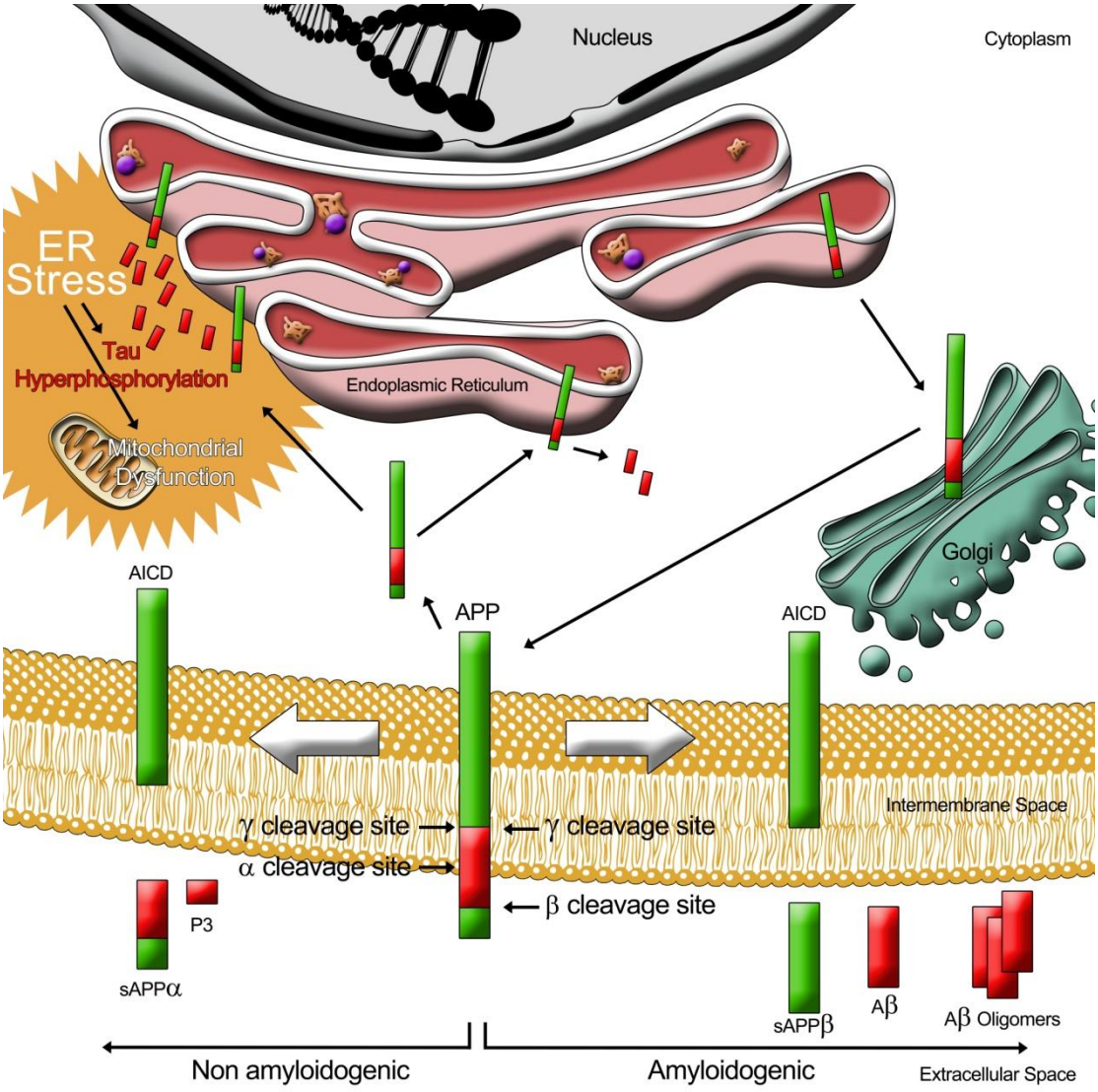

Figure 3

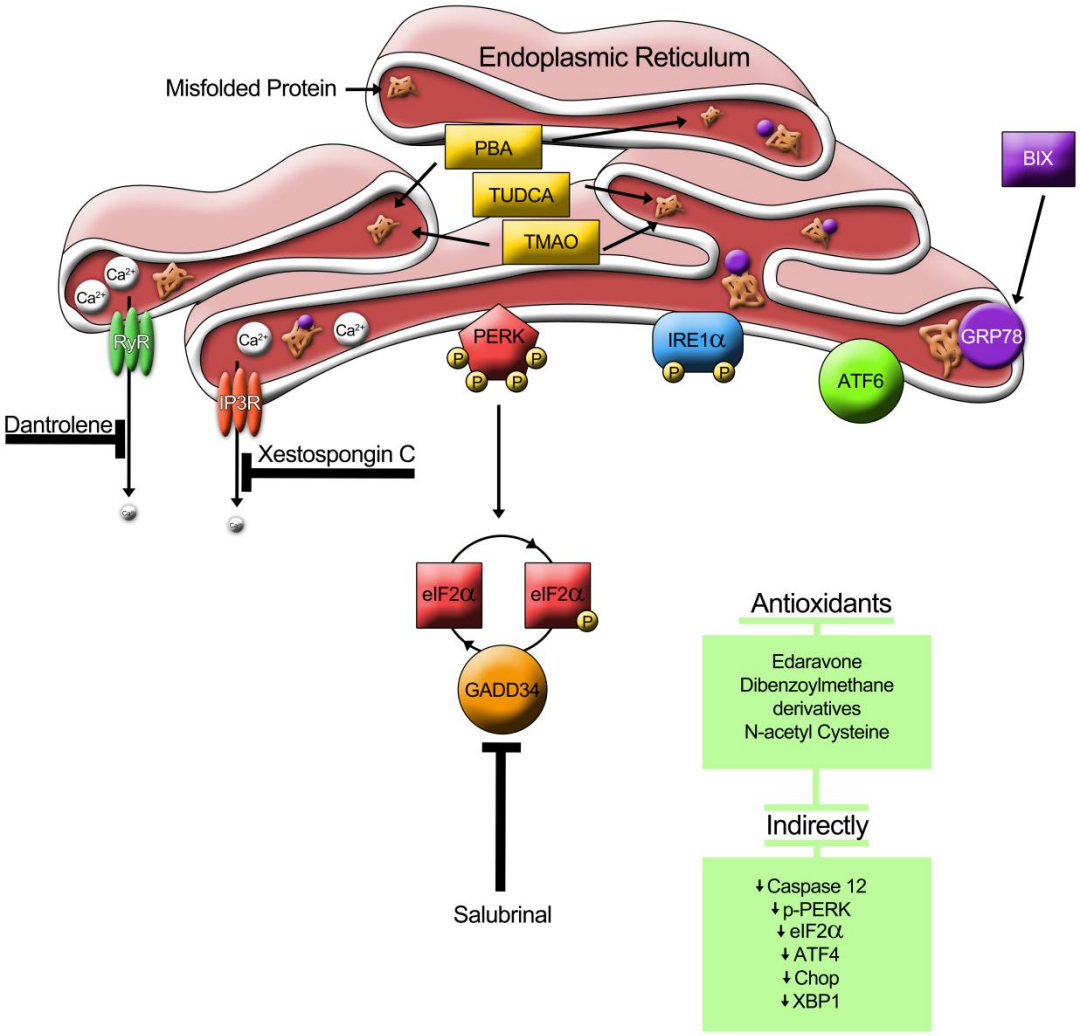

Figure 4

Highlights

- Endoplasmic reticulum (ER) is involved in the processing and trafficking of amyloid precursor protein (APP)
- Alzheimer's disease (AD) is characterized by endoplasmic reticulum (ER) stress
- ER is a potential therapeutic target in AD