



The role of the Amyloid Precursor Protein mutations and PERK-dependent signaling pathways in the pathogenesis of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a highly complex, progressive, age-related neurodegenerative human disease entity. The genetic basis of AD is strictly connected with occurrence of mutations in *Amyloid Precursor (APP)* gene on chromosome 21. Molecular mechanism that leads to AD development still remains unclear. Recent data reported that it is closely correlated with Endoplasmic Reticulum (ER) stress conditions, which subsequently activate Unfolded Protein Response (UPR) signaling pathways, via the induction of protein kinase RNA-like endoplasmic reticulum kinase (PERK), as a self-protective, adaptive response to adverse stress conditions. That results in the attenuation of global protein synthesis and, on the contrary, selective translation of Activating Transcription Factor 4 (ATF4) and secretase β . Interestingly, under prolonged, severe ER stress UPR may switch its signal into apoptotic cell death. That ensues by ATF4-CHOP-mediated activation of a range of pro-apoptotic genes and, on the other hand, downregulation of the expression of the *anti-apoptotic protein B-cell lymphoma 2 (Bcl-2)* genes. Current investigations suggest that inhibitions of PERK activity may contribute to the attenuation of the deposition of toxic senile plaques in the brain tissue and, as a result, prevent degeneration of neurons and decline in cognitive abilities.

KEY WORDS: Amyloid β , Endoplasmic Reticulum stress, Unfolded Protein Response, eIF2 α , CHOP

Introduction

Alzheimer's disease (AD) is a progressive, pathological, irreversible disease entity of the Central Nervous System (CNS) (Pedrini et al. 2009). AD, the most common type of dementia,

constitutes as a huge health problem and has a significant influence on society. Current estimates suggest that nowadays 24 million people worldwide suffer from dementia (Pennanen et al. 2004). AD

with the highest frequency affect individuals in advanced age, but it is not a rigid rule, since AD may also affect younger people before the age 65 (Guerreiro et al. 2012, Babusikova et al. 2011). Type of AD, that tends to develops in individuals under 65 years is termed early-onset AD (EOAD), but in patients over 65 years is known as a late-onset AD (LOAD) (Tang & Gershon 2003). Interestingly, the number of patients with dementia is steadily increasing. Estimates suggest that AD may affect 65.7 million of people by 2030 and 115.4 million by 2050 (Babusikova et al. 2011).

AD is strictly connected with numerous changes not only in anatomy of tissue brain, but also in its biochemistry, genetic and function (Babusikova et al. 2011). The general features of AD are closely associated with memory loss and impairment of cognitive skills. AD and other neurodegenerative entities such as prion and Parkinson's diseases are connected with the accumulation of the misfolded or unfolded proteins in the lumen of the ER, which evoke ER stress conditions. That elicits activation of the PERK kinase and, as a consequence, Unfolded Protein Response pathways, that constitutes as a pro-adaptive cellular program to cope with unfavourable stress conditions. Paradoxically, long-termed stress conditions and over-activation of the PERK-dependent signaling pathways switch the pro-adaptive cellular response to pro-apoptotic signaling pathway (Moreno et al. 2012). Above-mentioned process leads to synaptic failure and significant loss of brain mass in AD patients (Pennanen et al. 2004). The main cause of Alzheimer's disease still remains unclear, but several lines of evidence suggest that the core of the

problem lies in genetic disorder. Nowadays, available AD treatment is insufficient, since may only alleviate symptoms of AD. Due to that fact better understanding of the molecular mechanisms, that elicit cell death by apoptosis is a promising avenue on developing more effective AD treatments (Ballard et al. 2011).

Gene mutations and mechanisms involved in A β plaques aggregation

At the neuropathological level deposition of neurotoxic amyloid beta (A β) plaques in tissue brain as well as significant loss of neurons represent the main hallmark of AD (Kumar & Walter 2011). Senile plaques among the neurons in the brain are predominantly composed of A β peptide consisting of 39-42 amino acids, which is generated during Amyloid Precursor Protein (APP) processing. Longer form of A β consisting of 42 amino acids creates aggregates with higher frequency (Kumar & Walter 2011), since it is inherently more fibrillogenic as compared to the shorter form of A β 40 (Price et al. 1995). A β 40 is a predominant variant, which represent approximately 90% of all generated fragments of A β (Decock et al. 2016).

Numerous studies were undertaken to gather knowledge about the genetic basis of AD. *APP* on chromosome 21. is the first detected gene that is strictly connected with AD development (Tang & Gershon 2003). The product of the *APP* gene is one of the I transmembrane glycoprotein, that occurs in three different isoforms such as: APP695, APP751, APP770 amino acids (Belyaev et al. 2010). Proteolytic cleavage of the product of *APP* gene may occur via two different molecular pathways: amyloidogenic and non-amyloidogenic. During the first process a vital role plays

secretases β and γ , but during the second one α and γ (Ehehalt et al. 2003). Secretase β , also termed Beta-secretase 1 (BACE1), belongs to the family of aspartyl protease (Dislich & Lichtenthaler 2012) and, like APP, it is expressed in several areas of the brain. Interestingly, to confirm a fundamental role of BACE1 in AD pathogenesis scientists reported its increased level and enhanced activity in post mortem AD brains (Fukumoto et al. 2002, Harada et al. 2006). Furthermore, the level of secretase β and its biological activity are increased nearly twofold in AD tissue brain (Li et al. 2004). Secretase γ consists of a complex of proteins such as presenilin 1 (PS1) and presenilin 2 (PS2), nicastrin, anterior pharynx-defective 1 (Aph1) as well as presenilin enhancer 2 (Pen2) (Cole & Vassar 2007). The third one, secretase α , is the member of the ADAM 10 family of disintegrin metalloproteinase (Lichtenthaler 2012).

The physiological processing of APP occurs within the A β sequences, therefore precludes generation of full-length A β (Sisodia 1992). During this pathway first cleavage through secretase α , at the specific site K687/L688, releases two extracellular products such as N-terminal APP α and C-terminal fragment C83. The second one is processed via secretase γ that leads to the production of two smaller, non-amyloidogenic fragments: p7 and p3 (Vassar 2004). Amyloidogenic APP processing via secretase β occurs generally at the specific site such as M671/D672 and G680/Y681 (Vassar et al. 2009). That generates amino-terminal fragment APP β as well as membrane-associated C99, that is proteolytically cleaved by secretase γ . After the second cleavage toxic A β peptide and amino-terminal fragment termed APP

intracellular domain (AICD) is generated (Chow et al. 2010). Secretase γ is a specific enzyme that cleaves C99 fragment at sites that generates A β consisting of different number of amino acids such as: G708/G709 - A β 37; G709/V710 - A β 38; V711/I712 - A β 40; V713/I714 - A β 42 (Tian et al. 2010, Perez et al. 1999). Hence, secretase γ processing is fundamental for AD development, since it creates A β consisting of different number of amino acids, including its pathogenic, toxic form A β 42 (Vassar et al. 2009) (Fig. 1).

Chromosome 21. is known as the smallest human autosome. Mutations in 14 known genes localized on chromosome 21. are known as a major cause of numerous monogenic disorders (Hattori et al. 2000). There is a body of evidence suggesting that APP mutations constitute as one of the main cause of EOAD as well as Familial Alzheimer's disease (FAD) with autosomal dominant inheritance. That leads to rapid changes in brains' neurons and, as a consequence, pathological cleavage of APP, which cause amyloidosis via extracellular deposition of A β plaques among the neurons (Weggen & Behr 2012). Moreover, above-mentioned *APP* mutations lead to the aberrant cleavage of APP via specific secretases (Hardy 1997). It has been reported that the most common APP mutations are the London, Dutch, Swedish and Flemish among others (Tang & Gershon 2003) (Tab. 1). Mutations in *APP* gene, which are localized at the cleavage site especially for secretase β promote APP processing in amyloidogenic pathway, but mutations near the cleavage site for secretase γ cause increased generation of A β 42 with higher ability to creation of senile plaques in tissue brain (Zhou et al. 2011).

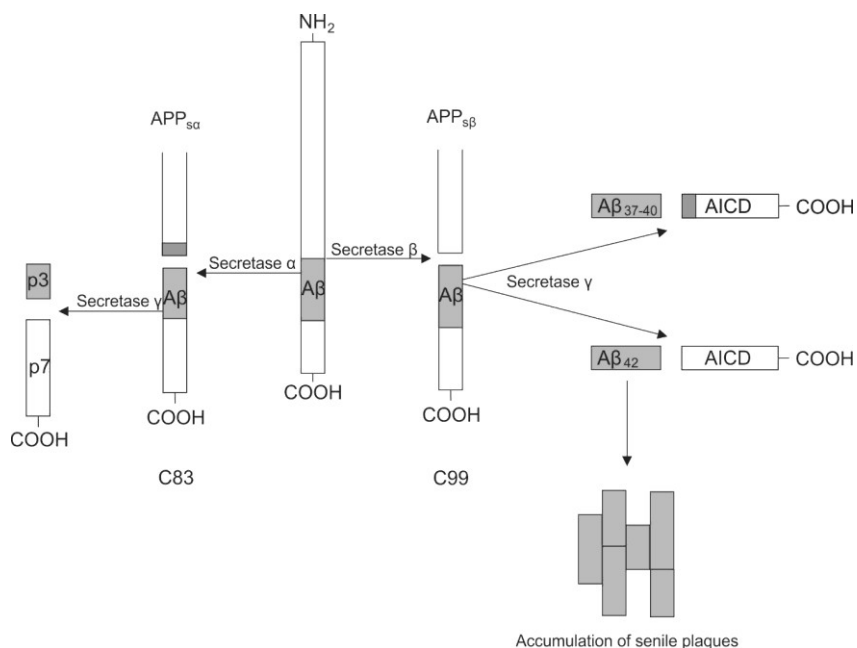


Figure 1. Molecular mechanisms of processing of the APP by α , β and γ secretases (A β – amyloid beta, APP – Amyloid Precursor Protein, APP_{sa} – soluble Amyloid Precursor Protein- α , APP_{sb} – soluble Amyloid Precursor Protein- β , AICD – Amyloid Precursor Protein intracellular domain, C83 – 83-amino-acid C-terminal fragment, C99 – 99-amino-acid C-terminal fragment, p3 – 3 kDa product, p7 – 3 kDa product).

Table 1. Examples of common APP mutations that promotes generation of A β ₄₂.

APP Mutation	Amino acid change	Site of APP mutation
Swedish	Lysine > Asparagine Methionine > Leucine	670 671
English	Histidine > Arginine	677
Tottori	Aspartic acid > Asparagine	678
Taiwanese	Aspartic acid > Histidine	678
Leuven (Italian)	Glutamic acid > Lysine	682
Flemish	Alanine > Glycine	692
Arctic	Glutamic acid > Glycine	693
Italian	Glutamic acid > Lysine	693
Dutch	Glutamic acid > Glutamine	693
Iowa	Aspartic acid > Asparagine	694
Austrian	Threonine > Isoleucine	714
Iranian	Threonine > Alanine	714
German	Valine > Alanine	715
French	Valine > Methionine	715
Florida	Isoleucine > Valine	716
Indiana	Valine > Phenylalanine	717
London	Valine > Isoleucine	717

ER stress activates PERK-dependent Unfolded Protein Response signaling pathways

The ER is a network of tubules and sacs that extend in the cell cytosol. ER plays a vital role in protein synthesis, post-translational modifications and protein folding. Moreover, ER provides proper biosynthesis of phospholipids and maintains calcium homeostasis (Rutkowski & Kaufman 2004, Cooper 2000). A range of stressful, pathological conditions have a significant influence on ER function. Stress stimuli include deprivation of nutrients, changes in redox homeostasis, increased level of protein synthesis, hypoxic conditions, viral infections as well as deficiency of calcium ions in the ER lumen (Pytel et al. 2014). It has been reported that one important role of ions calcium is to support functioning of ER chaperones and maintain proper protein folding. Current estimates have suggested that A β peptides trigger release of calcium ions toward cell cytoplasm. ER luminal depletion of calcium ions has a negative impact on chaperone activity as well as protein folding (Leissring et al. 2001). Disturbances in physiological functions of ER evoke ER stress, which activates PERK-dependent UPR signaling network known as a set of pro-adaptive signaling pathways, which the main aim is to restore ER homeostasis (Xu et al. 2005). Above-mentioned adaptive response involves enhanced expression of genes, which are responsible for proper protein folding within ER lumen as well as degradation of pathological proteins. Due to adverse conditions misfolded and unfolded proteins are accumulated within the ER lumen, and subsequently global protein synthesis is inhibited to reduce influx of a new, aberrant proteins into the ER lumen (Xu et al. 2005). Generally, native monomeric proteins possess α -helix confirmation, but the characteristic

feature of misfolded proteins is β -sheet confirmation. It allows to conclude that aggregation of aberrant proteins within the ER lumen is the main cause of neurodegenerative diseases (Doyle et al. 2011). Paradoxically, UPR has a dual role, since if during prolonged stress conditions the pro-survival response fails apoptotic cell death ensues (Vandewynckel et al. 2013). Interestingly, UPR is implicated in the pathogenesis of numerous human disease entities such as neurodegenerative diseases, including AD, cancer and a range of inflammatory diseases like atherosclerosis, type II diabetes, renal disease, arthritis as well as inflammatory bowel disease (Brown & Naidoo 2012, Tabas & Ron 2011).

The lumen of the ER is crowded with folding enzymes and chaperones such as immunoglobulin heavy chain-binding proteins (BiP), that play a major role in all stages of protein folding (Brown & Naidoo 2012). Under normal conditions they create a specific complex with inactive ER transmembrane receptor like PERK (Vandewynckel et al. 2013), which belongs to the serine/threonine protein kinase. During aggregation of misfolded and unfolded protein within the ER lumen BiP dissociate from receptors' catalytic domains and PERK undergoes oligomerisation and trans-autophosphorylation, that trigger its rapid activation (Harding et al. 1999, Ma & Hendershot 2002). Activated PERK subsequently phosphorylates Eukaryotic Initiation Factor 2 alpha (eIF2 α) at Ser51 (Doyle et al. 2011) resulting in significant attenuation of global protein translation and induction of translation of only selective mRNA such as secretase β , ATF4 and CCAAT-enhancer-binding protein homologous protein (CHOP), that may trigger cell death via apoptosis (Blais et al. 2004, Nishitoh 2012, Devi & Ohno 2014).

It has been reported that eIF2 consists of three major parts such as subunits: α , β and γ (Suragani et al. 2006). Translation initiation is strictly dependent on eIF2, since it possesses the ability to create a multiprotein complex with guanosine triphosphate (GTP) and initiator-methionyl-tRNA. Subsequently, that complex interacts with the smaller ribosomal subunit termed 40S, which results in creation of pre-initiation complex 43S, that is directly responsible for the initiation of protein translation (Kimball 1999). Above-mentioned complex binds to mRNA and moves downstream toward the initiation codon AUG. As a consequence of correct codon-anticodon pairing the 48S pre-initiation complex is formed. During that process energy is released, since GTP is hydrolyzed to GDP via GTP-ase-activating protein eIF5 (Elsby et al. 2011). Creation of a new ternary complex is closely connected with conversion of GDP to GTP, which is catalyzed by guanine nucleotide exchange factor eIF2 β . Interestingly, phosphorylation at Ser51 subunit α of eIF2 by activated PERK, under ER stress conditions, triggers inhibition of global protein synthesis, since exchange of GDP to GTP is abrogated. As a result formation of a new ternary complex and subsequent protein translation is effectively inhibited (Krishnamoorthy et al. 2001).

There is abundant evidence that the level of phosphorylated eIF2 α is significantly increased in AD brain tissue. Besides, currently many studies were undertaken to confirmed increased level of phosphorylated eIF2 α in transgenic AD mouse models with memory impairments such as 5XFAD and APP/PS1 KI. These studies have shown that aberrant activation of PERK kinase in AD brain may represent the main mediator of eIF2 α phosphorylation

at Ser51 in AD brains (Duran-Aniotz et al. 2014). Likewise, recent data have suggested that increased level of phosphorylated eIF2 α in tissue brain of AD patients and APP transgenic mice is accompanied with higher level of secretase β , that is responsible for the activation of A β production (Devi & Ohno 2014). It has been reported that monomers of A β become toxic for brains nervous tissue after their aggregation into oligomers, and then in higher aggregated conformations (Lorenzo & Yankner 1994, Pike et al. 1991).

Molecular mechanisms of ER stress-induced apoptosis

The crucial role of the activation of UPR signaling network is to rebalance ER homeostasis. Generally, UPR is known as a molecular mechanism by which ER copes with adverse, pathological conditions (Wagner & Moore 2011). During severe and long-termed stress conditions pro-adaptive signaling pathways of the UPR are insufficient. Persistent activation of ER transmembrane receptors and CHOP may evoke cell death via apoptosis (Szegezdi et al. 2006). The characteristic hallmark of CHOP is its expression at low concentrations during physiological conditions, but its synthesis is significantly elevated under prolonged stress conditions. Transcription of CHOP occurs when ER membrane-bound receptors, such as PERK, are in active state. Notably, PERK/eIF2 α /ATF4 signaling network, induced by ER stress, triggers markedly elevated expression of CHOP, that results in apoptotic cell death (Oyadomari & Mori 2004).

There is a body of evidence suggesting that ER stress-induced apoptosis is closely associated with human disease entities including neurodegenerative diseases (Tabas & Ron 2011), but the mechanism

responsible for switching pro-adaptive signaling branches into pro-apoptotic still remains unclear (Doyle et al. 2011). High level of phosphorylated eIF2 α , as a consequence of PERK activation, results in enhanced translation of ATF4. That stimulates expression of numerous genes responsible for adaptive response to adverse conditions (Schonthal 2012). On the contrary, during ER stress conditions, ATF4 as a transcription factor upregulates expression of pro-apoptotic *DNA Damage Inducible Transcript 3 (DDIT3)* gene that encodes CHOP protein (Dey et al. 2010). CHOP is involved in downregulation of the expression of the anti-apoptotic *Bcl-2* gene family and, adversely, upregulation the pro-apoptotic genes encoding proteins such as Bcl-2-like protein 11 (Bim), BH3 Interacting Domain Death Agonist (Bid), Phorbol-12-myristate-13-acetate-induced protein 1 (Noxa), p53 upregulated modulator of apoptosis (Puma) among others. Above-mentioned proteins are the members of the BH3 domain-only proteins and possess the ability to the induction of apoptosis via attenuation of biological activity of anti-apoptotic Bcl-2 proteins (McCullough et al. 2001, Shamas-Din et al. 2011).

Furthermore, one of the transcriptional target of CHOP is Growth arrest and DNA damage-inducible protein (GADD34), which may dephosphorylate eIF2 α , thus lead to

global translation recovery under non-physiological, stress conditions (Marciniak et al. 2004). Upon this negative feedback loop GADD34 directly combine with protein phosphatase 1 (PP1). That complex dephosphorylates eIF2 α , triggers translational recovery, thus promotes ER nascent protein loads, ER stress and, as a result, apoptotic cell death (Feldman et al. 2005, Brush et al. 2003).

CHOP also significantly increases expression of *ER oxidoreductin 1 α (ERO1 α)* genes. Products of that genes, under ER stress conditions, promotes a hyperoxidizing environment, that evokes cell death via apoptosis (Sevier & Kaiser 2008, Simmen et al. 2010). Additionally, CHOP, upon oxidized conditions, may also activate calcium-release channel inositol-1,4,5-trisphosphate receptor 1 (IP3R1). Finally, leakage of calcium ions into the cell cytoplasm activates calcium-sensing kinase termed calcium/calmodulin-dependent protein kinase II (CaMKII). That events results in the activation of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) subunit 2 (NOX2) and subsequent production of reactive oxygen species (ROS). That creates a positive feedback loop, since ROS generates by NADPH oxidase promotes expression of CHOP and, as a consequence, apoptotic cell death (Tabas & Ron 2011) (Fig. 2).

Conclusions

Dementia constitutes one of the primary health problem. Current data suggest that approximately 24 million people suffer from AD and 80% cases involve mutations in genes. The characteristic feature of AD is a deposition of senile plaques mainly composed of toxic form of A β consisting of 42 amino acids, which leads to neuronal loss and impairment of memory

function in AD patients. Despite, the molecular basis of AD is not fully understood, recent study has suggested that the core of the AD disorders lies in genetic factors. Mutations in *APP* gene on chromosome 21. activate amyloidogenic pathways, where an essential role, during generation of toxic A β 42, plays secretase β . Moreover, recent data have suggested that

disturbances on the molecular level are closely associated with ER stress an subsequent activation of PERK-dependent UPR signaling branches, that possess a dual role such as pro-adaptive and pro-apoptotic, which depends on the severity of stress conditions as well as exposure time of neurons to unfavorable factors.

Nowadays, only symptomatic treatments is available for cognitive decline in AD. Current data suggest that attenuation of PERK via its small-molecule inhibitors may prevents excessive phosphorylation of eIF2 α , thus block enhanced β -amyloidogenesis through significant decline in APP cleaving in amyloidogenic pathway, what seems to be important in future therapies.

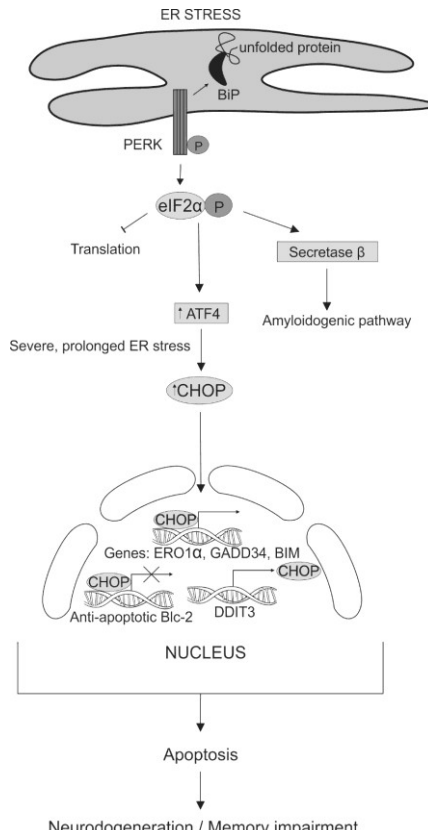


Figure 2. Mechanisms of the activation of the pro-adaptive response and CHOP-induced apoptosis under ER stress conditions (ER – Endoplasmic Reticulum, P – phosphate group, BiP - immunoglobulin heavy chain-binding proteins, PERK - protein kinase RNA-like endoplasmic reticulum kinase, eIF2 α - Eukaryotic Initiation Factor 2 alpha, ATF4 - Activating Transcriptor Factor 4, CHOP - CCAAT-enhancer-binding protein homologous protein , ERO1 α - ER oxidoreductin 1 α , GADD34 - Growth arrest and DNA damage-inducible protein, BIM - Bcl-2-like protein 11, Bcl-2 - protein B-cell lymphoma 2, DDIT3 - DNA Damage Inducible Transcript 3).

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Streszczenie

Choroba Alzheimera (ang. *Alzheimer's disease*, AD) jest przewlekłą, najczęściej występującą, chorobą neurodegeneracyjną prowadzącą do nieodwracalnych zmian w strukturze, biochemii i funkcjach mózgu. Neurodegeneracja Ośrodkowego Układu Nerwowego (OUN) jest wynikiem odkładania toksycznych złożeń amyloidu β ($A\beta$) w tkance nerwowej mózgu. Rozwój AD jest przyczyną skomplikowanych interakcji między podłożem genetycznym, a czynnikami biologicznymi, które aktywują złożone szlaki molekularne w przebiegu schorzenia. Za jedną z głównych przyczyn uważa się mutacje występujące w genie kodującym Prekursorowe białko amyloidu β (ang. *Amyloid beta Precursor Protein*, APP) zlokalizowane w pobliżu cięcia białka APP przez wysoce specyficzne sekretazy: α , β oraz γ . Generowanie toksycznej formy $A\beta$ o długości 42-óch aminokwasów, odkładanego w tkance mózgowej jako płytki starcze, zachodzi poprzez drogę amyloidogenną, w której uczestniczą sekretazy β oraz γ .

Na podłożu molekularnym główną przyczyną rozwoju choroby AD jest akumulacja błędnie sfałdowanych lub niesfałdowanych białek w lumen Retikulum Plazmatycznego (ang. *Endoplasmic Reticulum*, ER). Skutkuje to bezpośrednim wywołaniem stresu ER, który prowadzi do aktywacji kinazy PERK, a następnie fosforylacji eukariotycznego czynnika inicjacji translacji 2 (eIF2 α). W rezultacie w komórce nerwowej inhibowana jest translacja większości białek oraz dochodzi do preferencyjnej translacji wyłącznie takich białek takich jak ATF4 (ang. *Activating Transcriptor 4*) oraz, wyniku długotrwałych warunków stresowych, CHOP (ang. *CCAAT-enhancer-binding protein homologous protein*).

Nadekspresja białka CHOP prowadzi do wzmocnienia ekspresji genów kodujących: pro-apoptotyczne białka BH3 domain-only, GADD34 (ang. *DNA damage-inducible protein*, GADD34) oraz białko o aktywności oksydoreduktazy ER (ang. *ER oxidoreductin 1 α* , ERO1 α). W warunkach wysokiego stężenia białka CHOP zostaje osłabiona ekspresja genów kodujących anty-apoptotyczne białka Bcl-2. W rezultacie masa tkanki nerwowej mózgu ulega znaczącemu obniżeniu w wyniku postępującego procesu neurodegeneracji na drodze apoptotycznej śmierć komórkowej w przebiegu AD.