Biochimica et Biophysica Acta 1822 (2012) 885–896

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Oxidative stress increases BACE1 protein levels through activation of the PKR-eIF2α pathway

François Mouton-Liger [a,b,*], Claire Paquet [a,b,c], Julien Dumurgier [c], Constantin Bouras [d], Laurent Pradier [e], Françoise Gray [f], Jacques Hugon [a,b,c]

[a] Service d'Histologie et de Biologie du Vieillissement, APHP, Groupe Hospitalier Lariboisière Fernand-Widal Saint-Louis, Université Paris VII, Paris, France
[b] Inserm U839, Institut du Fer à Moulin, Paris, France
[c] Centre Mémoire de Ressources et de Recherche Paris Nord Ile de France, APHP, Groupe Hospitalier Lariboisière Fernand-Widal Saint-Louis, Université Paris VII, Paris, France
[d] Department of Neuropsychiatry, Geneva University Hospital, Geneva, Switzerland
[e] Sanofi-Aventis Therapeutic Strategy Unit Aging, 1 Avenue Pierre Brosselette 91385, Chilly-Mazarin, France
[f] Service d'Anatomo-Pathologie, APHP, Groupe Hospitalier Lariboisière Fernand-Widal, Saint-Louis, Université Paris VII, Paris, France

ARTICLE INFO

Article history:
Received 25 July 2011
Received in revised form 18 January 2012
Accepted 18 January 2012
Available online 28 January 2012

Keywords:
Alzheimer's disease
BACE1
PKR
eIF2α
Oxidative stress
Neuroblastoma cell cultures

ABSTRACT

Beta-site APP cleaving enzyme 1 (BACE1) is the rate limiting enzyme for accumulation of amyloid β (Aβ)-peptide in the brain in Alzheimer's disease (AD). Oxidative stress (OS) that leads to metabolic dysfunction and apoptosis of neurons in AD enhances BACE1 expression and activity. The activation of c-jun N-terminal kinase (JNK) pathway was proposed to explain the *BACE1* mRNA increase under OS. However, little is known about the translational control of BACE1 in OS. Recently, a post-transcriptional increase of BACE1 level controlled by phosphorylation of eIF2α (eukaryotic translation initiation factor-2α) have been described after energy deprivation. PKR (double-stranded RNA dependant protein kinase) is a pro-apoptotic kinase that phosphorylates eIF2α and modulates JNK activation in various cellular stresses. We investigated the relations between PKR, eIF2α and BACE1 in AD brains in APP/PS1 knock-in mice and in hydrogen peroxide-induced OS in human neuroblastoma (SH-SY5Y) cell cultures. Immunoblotting results showed that activated PKR (pPKR) and activated eIF2α (peIF2α) and BACE1 levels are increased in AD cortices and BACE1 correlate with phosphorylated eIF2α levels. BACE1 protein levels are increased in response to OS in SH-SY5Y cells and specific inhibitions of PKR-eIF2α attenuate BACE1 protein levels in this model. Our findings provide a new translational regulation of BACE1, under the control of PKR in OS, where eIF2α phosphorylation regulates BACE1 protein expression.

1. Introduction

Senile plaques, neurofibrillary tangles, synaptic and neuronal loss are salient neuropathological features of Alzheimer's disease (AD) [1]. Senile plaques consist mainly of amyloid β (Aβ) peptides generated after APP (amyloid precursor protein) proteolysis by the amyloidogenic pathway [2]. First, proteins possessing β-secretase activity cut APP, shedding the ectodomain and leaving in the membrane a fragment of 99 amino acids (C99) [3,4]. Afterwards, a γ-secretase complex cleaves C99, generating an Aβ peptide. According to the amyloid hypothesis [5], the accumulation and aggregation of Aβ is the trigger event leading to neurodegeneration in AD.

BACE1 (beta-site APP cleaving enzyme 1) is expressed in neurons and is responsible for most of the β-secretase activity in the central nervous system [6,7]. Previous studies have shown that BACE1 levels and activity are increased in postmortem AD brain samples [8-11], suggesting that elevated BACE1 levels might play a pivotal role in AD. BACE1 undergoes a complex set of post-translational modifications during its maturation. The immature form is cleaved by members of the Furin family of convertases [3]. This cleavage and the extent of *N*-glycosylation on 4 sites are required for efficient maturation [12]. BACE1 gene expression is tightly regulated at the transcription level by Sp1 [13] and STAT3 [14,15]. In AD brains, both normal [11,16] or increased [17,18] *BACE1* mRNA levels have been reported. However, if a transcriptional regulation could not be completely excluded, most studies indicate that a post-transcriptional control is mainly implicated in the increase of BACE1 detected in AD brains. Various hypotheses have been proposed to explain how BACE1 might be post-transcriptionally regulated in response to aging or pathological conditions including an alteration in specific microRNA profiles [19,20] and a caspase-3-dependent inactivation of GGA3 (Golgi-associated, gamma adaptin ear containing, ARF binding

* Corresponding author at: Histologie et Biologie du Vieillissement Groupe Hospitalier St Louis Lariboisière F.Widal, 2, rue Ambroise Paré. 75010 Paris, France. Tel.: +33 149956978, +33 145276151.
E-mail address: francois.mouton-liger@inserm.fr (F. Mouton-Liger).

0925-4439/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2012.01.009

protein 3)—mechanism leading to a decreased lysosomal degradation of BACE1 [21,22].

The double-stranded RNA-dependent protein kinase (PKR) is one of the four mammalian serine-threonine kinases (the three others being HRI (heme regulated inhibitor), GCN2 (general control non repressed 2) and PERK (PKR-like endoplasmic reticulum kinase)) that catalyzes the phosphorylation of the α subunit of eIF2 (eukaryotic translation initiation factor-2) in response to stress signals, leading to an inhibition of protein synthesis [23,24]. The activation of PKR is induced by various triggers such as viral infection, calcium overload, endoplasmic reticulum (ER) or oxidative stresses (OS) [25,26]. Recent studies have indicated that dsRNA-independent activation may be mediated by the overexpression of a direct activator: the human protein PACT (PKR ACTivating protein), or its mouse homologue, RAX (PKR-associated protein X) [27-30]. In AD brains, previous reports have shown that phosphorylated PKR is increased in neuronal cytoplasm and nuclei, in granulovacuolar degenerations, as well as around senile plaques [31,32]. Our previous study in 2002 has also demonstrated that phosphorylation of eIF2α could play an essential role in AD neurodegeneration [33]. These results suggest that PKR-induced cellular stress could contribute to neurodegeneration in AD [34]. PKR could be also involved in the mechanism of tau phosphorylation via Glycogen synthase kinase 3β (GSK-3β) phosphorylation [35].

Recently, a study has shown that energy deprivation induced PERK activation, the phosphorylation of eIF2α and an increased BACE1 translation [36]. Similarly, in the brains of the transgenic mouse 5XFAD with human APP mutation and an aggressive amyloid pathology, phosphorylation of eIF2α increases BACE1 levels and the abilities to control Aβ accumulation during the course of disease progression [37]. These alterations of *BACE1* mRNA translational control by eIF2α could be explained by a stress-dependent phenomenon of translation initiation. The *BACE1* mRNA 5′-untranslated region (5'UTR) acts as a translational repressor, characteristics shared with many 5′UTRs of transcripts that are translationally controlled by cellular stress, such as huntingtin [38] and transcription factors [39,40]. The presence of long, G-C-rich, upstream open reading frames (uORFs) in the 5′UTR is thought to down-regulate the efficiency of translation initiation of the main BACE1 ORF [41-43].

Post-mortem observations of AD brains provided strong evidences for increased levels of cellular OS [44-47]. There is also a significant correlation between BACE1 activity and oxidative markers in sporadic AD brains [48]. Previously, the activation of c-jun N-terminal kinase/activator protein 1 (JNK/AP1) pathway was proposed to explain the *BACE1* mRNA increase detected under OS [49]. However, little is known about the translational control of BACE1 in OS condition. The goal of this study was to assess the role of PKR in BACE1 protein expression during OS. Our results showed that $pPKR_{Thr446}$/PKR and $peIF2\alpha_{Ser51}$/peIF2α ratios and BACE1 levels are increased in AD brains and in APP/PS1 mouse brains. Furthermore, in neuroblastoma cells, OS induces the PKR-eIF2α pathway, and leads to an increase of BACE1 protein level. Moreover, the specific inhibition of PKR by chemical inhibition or by siRNA significantly attenuates BACE1 level in this cellular model.

2. Materials and methods

2.1. Human brains

For all human brains, consents were obtained for using postmortem tissues. The post-mortem intervals (PMI) never exceeded 24 hours. Assessment of pathology associated with dementia was performed by examining histological sections of frontal, temporal, parietal and occipital lobes, corpus striatum, thalamus, midbrain, pons, medulla and cerebellum. Selected sections were immunostained for Aβ, tau and α-synuclein. All these patients had a history of progressive dementia and satisfied National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders (NINCDS-ADRDA) criteria for probable AD and satisfied neuropathologic criteria for AD according the CERAD protocol.

For immunoblotting analysis, frozen brain samples of frontal cortex from 10 AD patients and 10 age-matched controls and temporal cortex from 8 AD and 7 age-matched controls were provided by the Department of Neuropsychiatry, University of Geneva, Geneva, Switzerland (Pr Constantin Bouras) (Table 1).

2.2. Mouse brains

All animal experiments were performed in accordance with the guidelines of the French Agriculture and Forestry Ministry for handling animals (decree 87849, license A75-05-22). All transgenic mice and littermate were provided by Sanofi. This APP/PS1 knock-in (APP/PS1 KI) transgenic mouse model carries M233T/L235P knocked-in mutations in presenilin-1 and overexpresses mutated human Aβ amyloid precursor protein Aβ x-42 is the major form of Aβ species present in this model with progressive development of a complex pattern of N-truncated variants and dimers, similar to those observed in AD brain. Furthermore, at 10 months of age, they display an extensive neuronal loss (>50%), which is already detectable at 6 months of age [50].

For brain preparation, three mice per genotype were anaesthetized with pentobarbital (40 mg/kg, i.p.) and received an intracardial perfusion with cold PBS. Brains were removed and dissected then placed in 4% (w/v) paraformaldehyde in PBS for immunohistochemistry. Brains homogenates of 9 months old transgenic mice APP/PS1 KI ($n=6$) and 4 age-matched wild type were established as described in a previous report [50].

2.3. Cell cultures

SH-SY5Y human neuroblastoma cells, obtained from the ATCC, were maintained at 37 °C under 5% CO_2/95% humidified air incubator in completed media with 45% Minimum Essential Media (MEM) (Invitrogen, Carlsbad, CA, USA) 45% Ham's F-12 medium (Invitrogen), 10% inactivated fetal bovine serum, enriched with 2 mM L-glutamine, 1 mM non essential amino acid (Invitrogen), 1 mM sodium pyruvate (Invitrogen), 100 U/ml penicillin and 100 mg/ml streptomycin

Table 1
This Table shows epidemiological and neuropathological characteristics of AD patients and controls brains for immunoblotting. The number of the patients, sex, age, post-mortem interval (PMI) and Braak stages are mentioned. Temporal cortex samples were correspond to patients and controls labeled with *. NA: Not available.

Patients	Age	Sex	PMI	Braak
AD1*	79	F	3	6
AD2*	85	F	5	5
AD3	95	M	8	5
AD4*	92	F	17	5
AD5	87	M	7	5
AD6*	83	M	12	NA
AD7*	102	F	4	5
AD8*	73	F	6	6
AD9*	86	F	8	5
AD1O*	73	M	6	5
Control 1*	80	F	10	1
Control 2	84	F	7	2
Control 3	97	F	8	2
Control 4	93	F	6	2
Control 5*	82	M	5	2
Control 6*	78	F	12	1
Control 7*	69	F	11	1
Control 8*	69	M	5	2
Control 9*	59	F	4	2
Control 10*	86	M	9	1

(Invitrogen). SH-SY5Y cells were grown to 90% confluence, and then treated with different chemical compounds.

2.4. Cell treatment

To induce oxidative stress, the cells were treated with hydrogen peroxide (H_2O_2) (Calbiochem, Darmstadt, Germany) at the concentrations 0.2 mM, and 0.5 mM from 30 minutes to 16 hours.

To inhibit the activation of PKR, cells were pretreated 1 hour before H_2O_2 exposure with PRI (peptide PKR inhibitor). PRI is coupled to a penetratin and can bind to the double-stranded RNA binding site of PKR and prevent its activation (NeoMPS Polypeptide Laboratories, Strasbourg, France) at various concentrations (50 to 100 μM). Due to the short-time effect, PRI treatments were repeated every hour.

The small-molecule drug salubrinal (Calbiochem) selectively blocks dephosphorylation of eIF2α by inhibiting the PP1 (Protein Phosphatase 1)/GADD34 (Growth Arrest and DNA Damage-Inducible Protein 34) complex formation [51]. Salubrinal was added to the cell media at 100 mM, 24 hours before stress exposure.

Cycloheximide (Chx) (Sigma, St. Louis, MO, USA), a protein synthesis inhibitor that acts specifically on the 60S subunit of eukaryotic ribosomes was used at 10 μg/ml to block protein synthesis in SH-SY5Y cells. To block transcription, Actinomycin D (ActD) (Calbiochem), an antineoplastic antibiotic known to act as a specific inhibitor of DNA-dependent RNA synthesis, was added to the cell media at 1 mg/ml. Finally, to selectively inhibit the proteasome, cells were pretreated with Lactacystin (Lact) (10 μM) (Sigma). When necessary, control cells were treated with DMSO.

2.5. Cell transfection

To selectively inhibit the PACT-PKR pathway, SH-SY5Y cells were transfected either with Short hairpin (Sh) RNA to PACT or silencing (si) RNA to PKR. Control (scrambled) siRNA (ScrPKR) and siRNA to PKR (5′-GCAGGGAGUAGUACUUAAAUAUU-3′) (SiPKR) were synthesized by Dharmacon Research, Inc. (Lafayette, CO, USA). The siRNAs (final concentration 25 nM) were transfected for 48 hours using FuGENE HD (Roche, Penzberg, Germany) before any treatment. ShRNA of PACT (ShPACT) (5′-GAACCAGCUUAAUCCUAUU-3′) and scrambled (ScrPACT) were transfected into SH-SY5Y cells using Lipofectamine 2000 (Invitrogen) for 48 hours before any treatment.

2.6. Tissue and cell extracts

Cortices were homogenized with a Potter in Laemmli sample buffer 1:10 (wt/vol) containing 5% sodium dodecyl sulfate (SDS), protease and phosphatase inhibitors cocktail. The lysate was sonicated and then centrifuged at 15,000*g* for 15 minutes at 4 °C.

To prepare cell lysates for immunoblotting, cells were rapidly washed in phosphate-buffered saline (PBS) and then lysed in a radio immune precipitation assay buffer (RIPA buffer) containing 25 mM β-glycerophosphate, 50 mM sodium fluoride, 2 mM sodium pyrophosphate, protease inhibitors (Roche), 1 mM sodium orthovanadate, and 0.1 mM calyculin (Sigma) as phosphatase inhibitors. They were sonicated and centrifuged at 20,000*g* for 10 minutes. The protein concentration in the supernatant was determined with Micro BCA Protein Assay Reagent Kit (Thermo scientific, Cergy-Pontoise, France) using the manufacturer's protocol.

2.7. Antibodies and immunoblot analysis

Protein samples (20 to 30 μg for brains samples, 50 μg for cell samples) separated on 4–12% (or on 12%) NuPAGE Bis-Tris gels (Invitrogen) and then electroblotted onto nitrocellulose membranes (GE Healthcare, Chalfont St. Giles, UK) at 400 mAmps per gel in 25 mM Tris, (pH 8.3), 200 mM glycine and 20% ethanol. After protein transfer, nitrocellulose membranes were blocked in 5% milk in TBS, and then incubated with primary antibody.

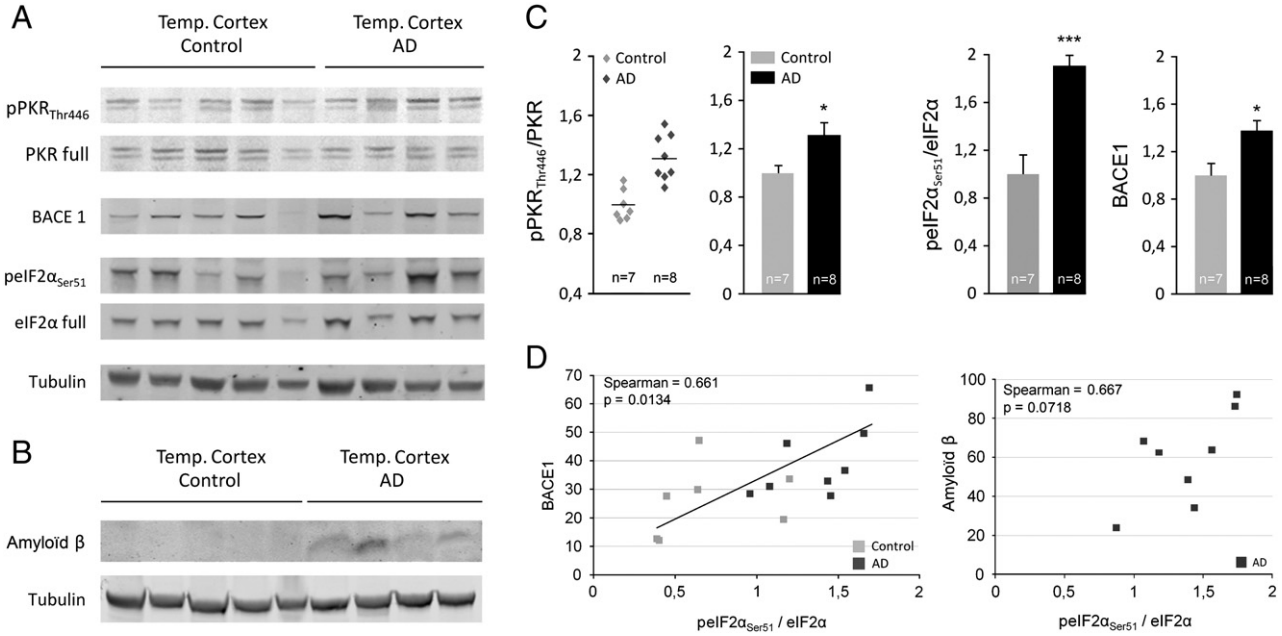

Fig. 1. Compared levels of $pPKR_{Thr446}$, PKR full, $peIF2\alpha_{Ser51}$, eIF2α full, BACE1 and amyloid β in post-mortem temporal cortex of AD patients ($n=8$) and controls ($n=7$). A. Immunoblot analysis of $pPKR_{Thr446}$, PKR full, $peIF2\alpha_{Ser51}$, eIF2α full and BACE1 in AD and control temporal cortex samples. B. Immunoblot analysis of amyloid β in AD and control temporal cortex samples. C. Histograms showing that $pPKR_{Thr446}$/PKR ratio, $peIF2\alpha_{Ser51}$/eIF2α ratio, and BACE1, normalized on tubulin level, are increased in AD temporal cortex. D. $peIF2\alpha_{Ser51}$/eIF2α ratio is correlated to BACE1 level but not to amyloid β in frontal cortex samples. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

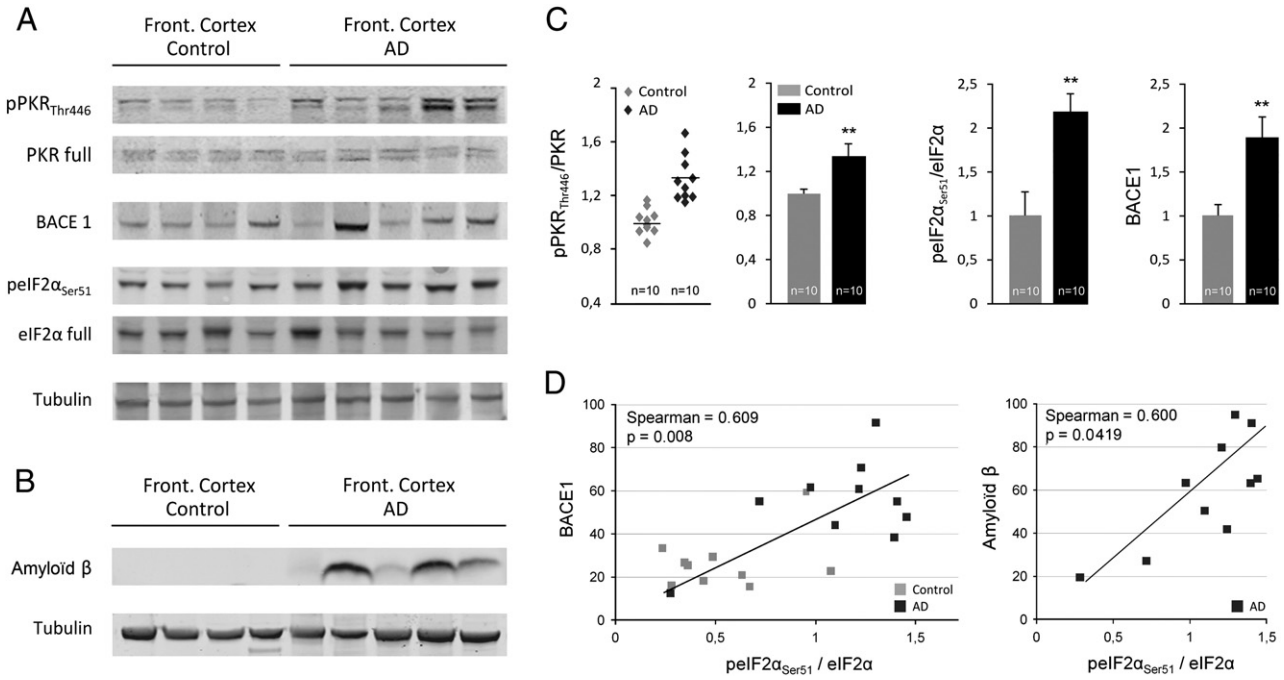

Fig. 2. Levels of pPKR$_{Thr446}$, PKR full, peIF2α$_{Ser51}$, eIF2α full, BACE1 and amyloid β in post-mortem frontal cortex of AD patients ($n=10$) and controls ($n=10$). A. Immunoblot analysis of pPKR$_{Thr446}$, PKR full, peIF2α$_{Ser51}$, eIF2α full and BACE1 in AD and control frontal cortex samples. B. Immunoblot analysis of amyloid β in AD and control frontal cortex samples. C. Diagram representations showing that pPKR$_{Thr446}$/PKR ratio, peIF2α$_{Ser51}$/eIF2α ratio and BACE1, normalized on tubulin level, are significantly increased in AD frontal cortex. D. Graphic representation that illustrates a significant correlation between peIF2α$_{Ser51}$/eIF2α ratio and BACE1 and amyloid β levels. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

The following primary antibodies were used: Mouse anti-Amyloid β (Millipore, Billerica, MA, USA), Rabbit anti-BACE1 (Santa Cruz, Danvers, MA, USA), rabbit anti-phosphorylated PKR$_{Thr446}$ (pPKR$_{Thr446}$) (Santa Cruz), rabbit anti-PKR (Cell Signaling, Beverly, MA, USA), goat anti-PACT (Santa Cruz), rabbit anti-phosphorylated PERK$_{Thr981}$ (pPERK$_{Thr981}$) (Santa Cruz), rabbit anti-PERK (Santa Cruz), rabbit anti-phosphorylated eIF2α$_{Ser51}$ (peIF2α$_{Ser51}$) (Cell Signaling), rabbit anti-eIF2α (Cell Signaling), mouse anti-Tubulin (Santa Cruz).

IR Dye 700DX conjugated anti-mouse IgG, IR Dye 800CW conjugated anti-goat IgG and IR Dye 800CW conjugated anti-rabbit IgG (Rockland Immunochemical Inc., Gilbertsville, PA, USA) were used as secondary antibodies.

Bound proteins were visualized with the Odyssey Imaging System (Li-Cor Biosciences, Lincoln, NE, USA) and quantified with Multigauge software (Fuji film, Tokyo, Japan).

2.8. Quantitative RT-PCR

Total RNA was isolated using TRIzol reagent (Invitrogen) from SH-SY5Y cells. SuperScript II reverse transcriptase kit (Invitrogen) with random decamer priming was used to synthesize the first strand cDNA from samples with an equal amount of total RNA (1 μg), according to the manufacturer's instructions. cDNA samples were forwarded to amplification with specific primers for PKR, BACE1 and GAPDH

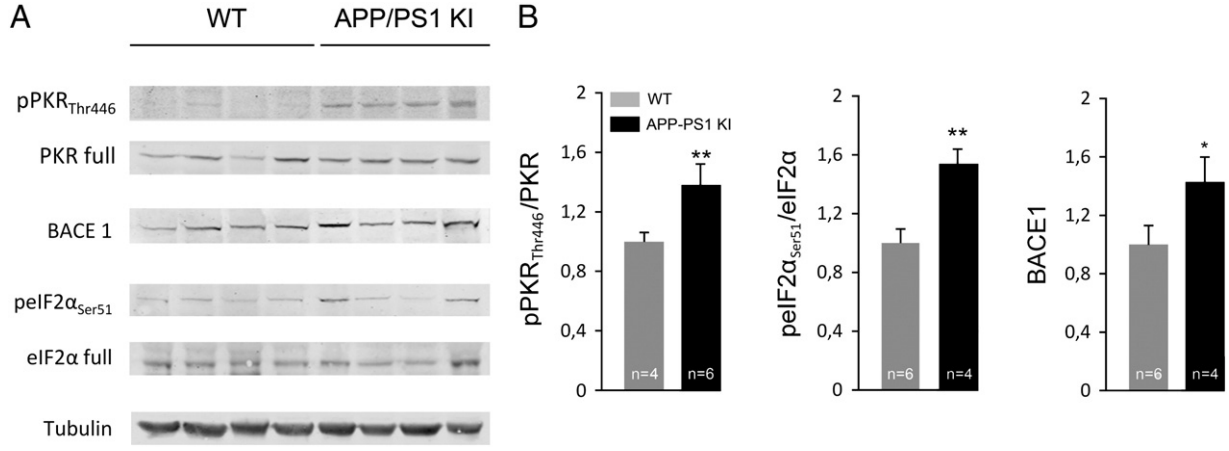

Fig. 3. Expression of pPKR$_{Thr446}$, peIF2α$_{Ser51}$ and BACE1 in post-mortem APP/PS1 KI mice and littermates (WT). A. Immunoblotting studies of mouse brains showed that pPKR$_{Thr446}$, peIF2α$_{Ser51}$ and BACE1 were increased compared to wild type brains. B. Diagram representations show that pPKR$_{Thr446}$/PKR ratio, peIF2α$_{Ser51}$/eIF2α ratio, and BACE1 are increased in APP/PS1 KI mice.

using SYBR Green technology (Invitrogen) and the Stratagene Mx3005P PCR cycler (Agilent Technologies, Massy, France). Human BACE1 primers [forward 5′-GCAGGGCTACTACGTGGAGA-3′ and reverse 5′-GTATCCACCAGGATGTTGAGC-3′], human GAPDH primers, [forward 5′-AATCCCATCACCATCTTCC-3′ and reverse 5′-GGACTCCAC-GACGTACTCA-3′] and human PKR primers [forward 5′-GGCACCCA-GATTTGACCTTC-3′ and reverse 5′-TCCTTGTTCGCTTTCCATCA-3′] were used in the present studies.

cDNA amplification was carried out as follows: denaturation at 94 °C for 2 minutes, followed by 35 cycles of denaturation at 94 °C for 15 seconds, primer annealing at 62 °C for 30 seconds, and extension at 72 °C for 40 seconds. A final extension was carried out at 72 °C for 10 minutes, ending with a 4 °C hold cycle. All assays were performed in triplicate, and BACE1 and PKR mRNA levels were normalized with the expression levels of GAPDH. Relative levels and gene copy numbers were calculated using the deltaCp method, as described by Pfaffl [52].

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA 92037 USA). Results were considered significant when $p<0.05$ using a non-parametric Mann and Whitney test. The correlation between PKR, BACE1 and eIF2α levels

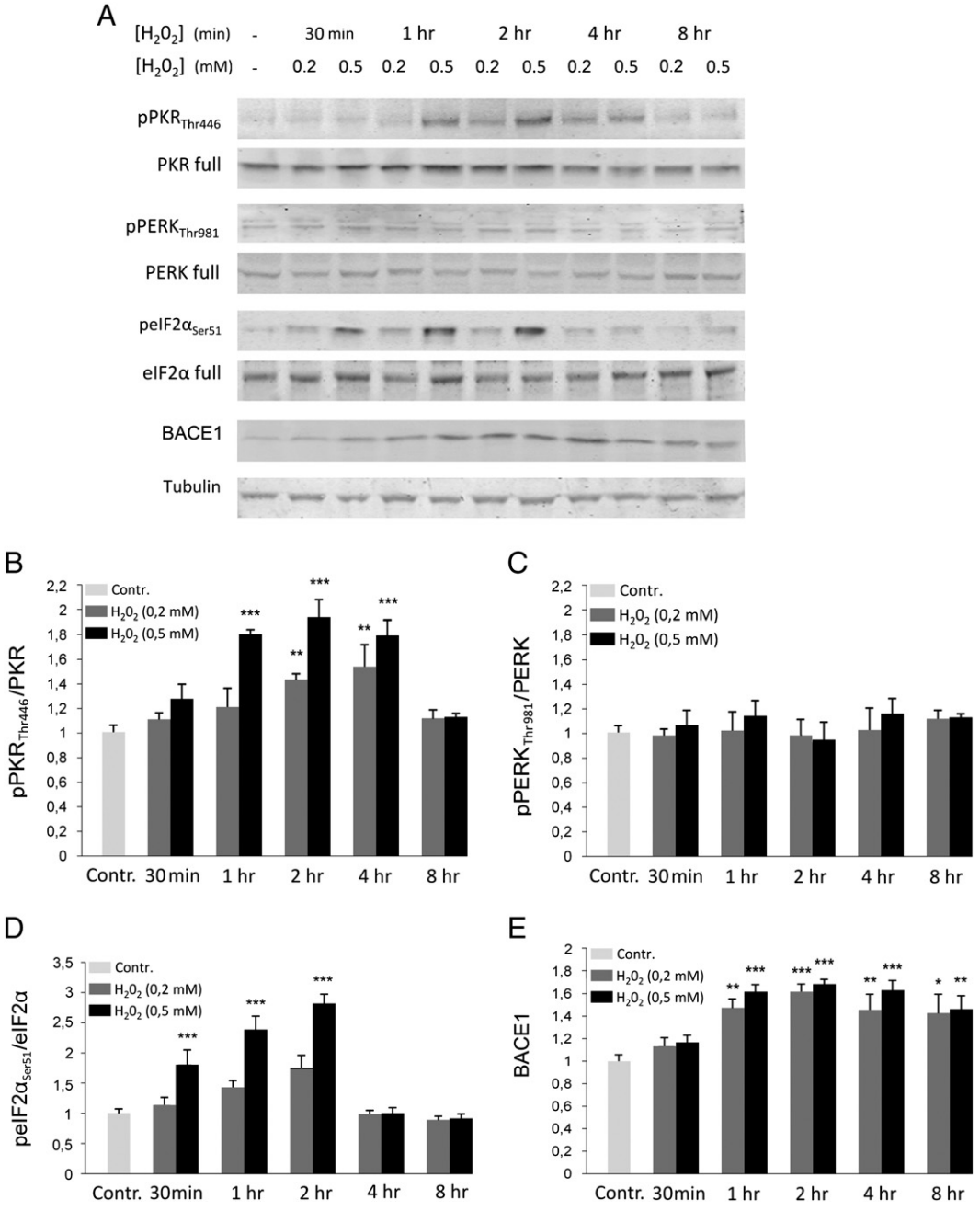

Fig. 4. Oxidative stress induced by H_2O_2 exposure in SH-SY5Y cells increases PKR, and eIF2α activations and BACE1 levels. Western blot of SH-SY5Y proteins extract revealed by eight different antibodies specific for $pPKR_{Thr446}$, PKR full, $pPERK_{Thr981}$, PERK, $peIF2\alpha_{Ser51}$, eIF2α full, BACE1 and tubulin. B–E. Histogram representation showing progressive time course enhancement relative to Tubulin level, respectively of $pPKR_{Thr446}$/PKR ratio (B), $pPERK_{Thr981}$/PERK ratio (C), $peIF2\alpha_{Ser51}$/eIF2α ratio (D), and BACE1 (E) after H_2O_2 exposure. $n=5$ for each condition. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

in cortical samples was assessed using Spearman correlation coefficients, in AD patients and control brains.

3. Results

3.1. Significant correlation between BACE1 levels and eIF2α activation in human AD brains

Immunoblot analysis of Fig. 1 revealed a statistically significant increase of amyloid β, BACE1 levels and peIF2α_{Ser51}/eIF2α ratio (60%) in AD ($n=8$) versus control temporal cortices ($n=7$) (Fig. 1A–B). Linear regression analysis showed a significant correlation between peIF2α_{Ser51}/eIF2α ratio and BACE1 levels, but not with amyloid β (Fig. 1D). We also assessed the activation of PKR in the temporal cortex, by measuring pPKR$_{Thr446}$/PKR ratio. PKR activation was increased in temporal AD cortices (30.7%) (Fig. 1A and C). The variations of phosphorylated forms of PKR and eIF2α proteins are correlated (data not shown), but linear regression method did not reveal a significant correlation between pPKR$_{Thr446}$/PKR ratio and BACE1 level (Fig. 1C).

In frontal cortex of AD patients ($n=10$), we also found increased eIF2α activation (112%), compared to controls ($n=10$) (Fig. 2A–C). These levels were significantly correlated in controls and AD patients with BACE1 levels (75%) and with amyloid β (Fig. 2B–D). Nevertheless, no significant correlation was observed between PKR activation (31.8%) (Fig. 2A and C) and BACE1 levels or amyloid β (data not shown).

3.2. Increases of BACE1 levels and eIF2α activation in APP/PS1-KI

Comparable analyses were performed in the APP/PS1 KI mice previously characterized by widespread neuronal degeneration and pPKR accumulation [53]. Molecular evaluations revealed increased pPKR$_{Thr446}$/PKR and peIF2α_{Ser51}/peIF2α ratios, respectively 39.6% and 58.4% (Fig. 3A and B) in APP/PS1 KI mice compared to control mice. In addition, the levels of BACE1 in transgenic mice were also increased by 43.2% (Fig. 3A and B). However, statistical analyses did not reveal significant correlation between PKR and eIF2α activation and BACE1 levels in this model.

3.3. OS induces an increase of PKR level and activation in SH-SY5Y cells

To assess *in vitro* if OS exposure could reproduce human findings, we treated neuroblastoma cells with H_2O_2 and analyzed by immunoblot PKR, pPKR$_{Thr446}$, eIF2α, peIF2α_{Ser51}, PERK, pPERK$_{Thr981}$ and BACE1 levels. In these cell extracts, a progressive enhancement of pPKR$_{Thr446}$/PKR and peIF2α_{Ser51}/eIF2α ratio (Fig. 4A–B and D) was

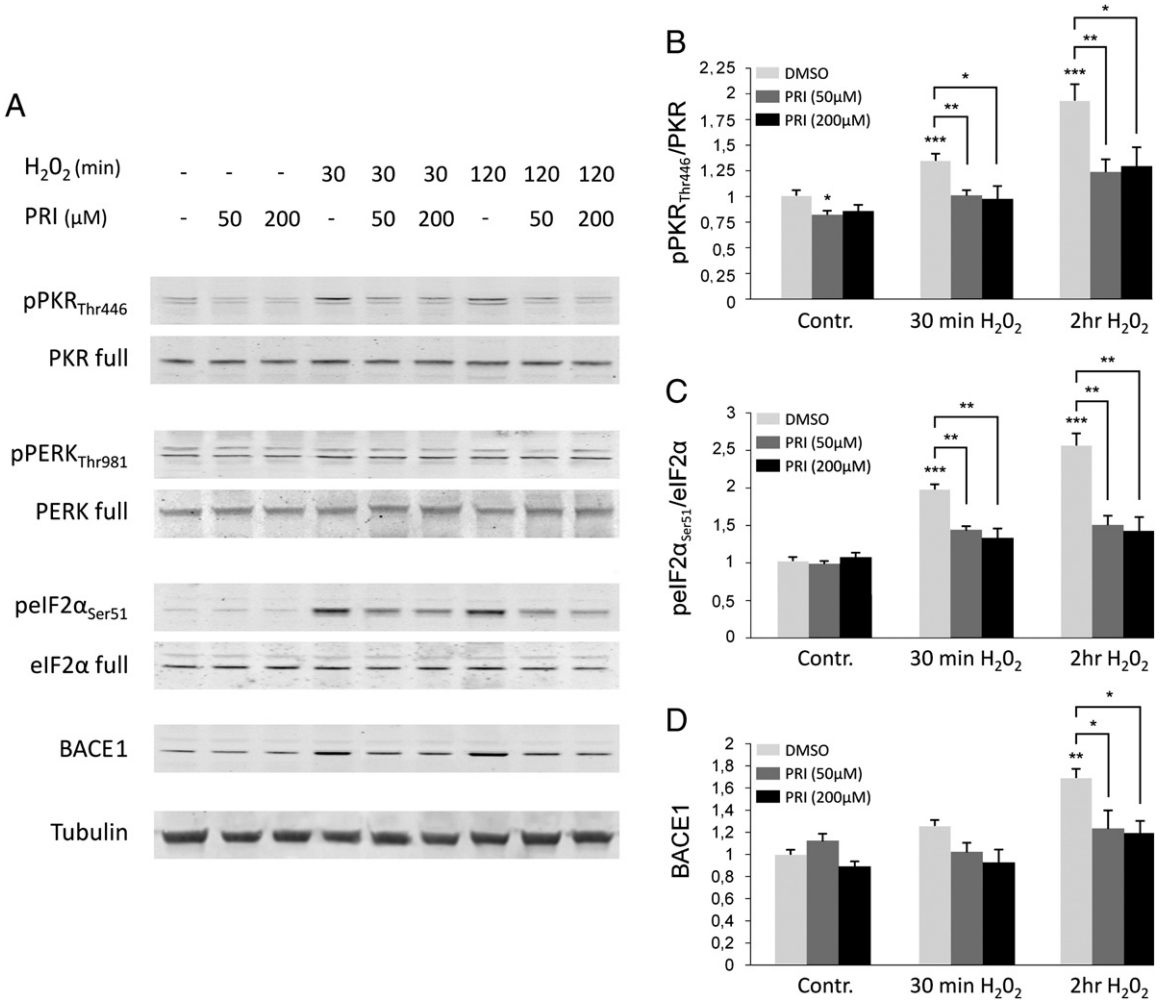

Fig. 5. Inhibition of PKR during H_2O_2-induced stress decreases eIF2α activation and BACE1 level. A. Western blot of SH-SY5Y proteins revealed by different antibodies specific for pPKR$_{Thr446}$, PKR full, pPERK$_{Thr981}$, PERK full, peIF2α_{Ser51}, eIF2α full, BACE1 and tubulin. B–D. Quantification of the relative level of pPKR$_{Thr446}$/PKR ratio (B); peIF2α_{Ser51}/eIF2α ratio (C), and BACE1 (D), normalized on Tubulin level, in SH-SY5Y treated with H_2O_2 (0.5 mM) and PRI (50 μM or 200 μM), compared to control cells (DMSO). n = 5 for each group. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

detected. Levels of phosphorylated PKR and eIF2α, reached a peak at 2 hours (respectively a 1.93 and 2.82 fold increase), and this changes were not anymore observed after 4 hours of treatment. In contrast, $pPERK_{Thr981}$/PERK ratio was unchanged (Fig. 4A and C). Finally, a transient increase of BACE1 level was detected with various concentrations of H_2O_2 (Fig. 4E). Notably, significant increases of BACE1 were found for the mature form of the protein. Immature forms of the protein have not been detected by our immunoblot approach. We therefore only present data for the mature BACE1 in figures and immunoblot analyses.

Moreover, in SH-SY5Y cells, PKR protein level progressively increased with 0.2 mM and 0.5 mM H_2O_2 treatment, reaching a maximum (+58.3%) at 2 hours (Suppl. Fig. 1A). Quantitative RT-PCR confirmed that OS leads to significant increase of *PKR* mRNA expression at 1 and 2 hours, respectively 32.3% and 26.8% (Suppl. Fig. 1B).

3.4. PKR inhibition reduces eIF2α activation and BACE1 level

To test if under OS condition PKR could control eIF2α phosphorylation and BACE1 expression, we pretreated SH-SY5Y cells with PRI, a specific inhibitor of PKR activation (Fig. 5). Using immunoblot analysis, we showed that PRI inhibits PKR activation in physiological conditions and partially reversed OS effect on $pPKR_{Thr446}$/PKR ratios after H_2O_2 exposure (30 minutes and 2 hours) (Fig. 5A–B). PRI did not affect PERK activation. PRI addition also significantly inhibited eIF2α activation, induced by H_2O_2, starting after 30 minutes of treatment (Fig. 5A and C). BACE1 expression was also reduced by PRI after 2 hours of H_2O_2 exposure, indicating that PKR could control BACE1 levels during OS (Fig. 5A and D).

To confirm these results, we evaluated the effect of PKR siRNA (SiPKR) in our system. SH-SY5Y cells transfected with SiPKR were exposed to 0.5 mM of H_2O_2. Immunoblot analysis (Fig. 6A), showed a reduction of PKR, $peIF2\alpha_{ser51}$/eIF2α ratio and BACE1 level in SiPKR-transfected cells compared with non-transfected cells or in cells transfected with a scramble siRNA-negative control of PKR (ScrPKR) (Fig. 6B–C).

To determine the implication of the PKR activator (PACT) in this regulatory pathway, we also used short-hairpin RNA of PACT. Western blot experiments on SH-SY5Y cells transfected with ShPACT revealed that the inhibition of PACT expression, attenuated BACE1 level enhancements (−20%) induced by OS (Fig. 6B–C). These findings add gene expression evidence that the PACT-PKR pathway can regulate BACE1 expression levels in our cell culture models.

3.5. Activation of eIF2α is implicated in OS-induced BACE1 increase

Correlation observed in frontal and temporal cortices between $peIF2\alpha_{Ser51}$/eIF2α ratio and BACE1 suggest that *BACE1* mRNA translation could be enhanced by eIF2α activation. To directly test this hypothesis, we exposed neuroblastoma cells, 24 hours before H_2O_2 treatment, to salubrinal (Sal), a drug which increases phosphorylated eIF2α (Fig. 7). As expected, Sal treatment leads to a significant increase (30.7%) of $peIF2\alpha_{Ser51}$/eIF2α ratio in OS condition (Fig. 7C). BACE1 levels induced by OS, were also increased (+20.2%) by salubrinal exposure (Fig. 7D), whereas PKR activation was not modified by salubrinal (Fig. 7B).

3.6. OS-induced BACE1 increases at transcriptional and translational levels

To determine if translational regulation by the PKR-eIF2α pathway is the only cause of OS-induced BACE1 elevation, we successively exposed cells to inhibitors of translation, transcription, and proteasomal activity during OS.

Firstly, we validated our hypothesis for the translational control of BACE1 under OS by treating SH-SY5Y cells with cycloheximide, an inhibitor of protein biosynthesis. After 2 hours of Chx treatment, BACE1

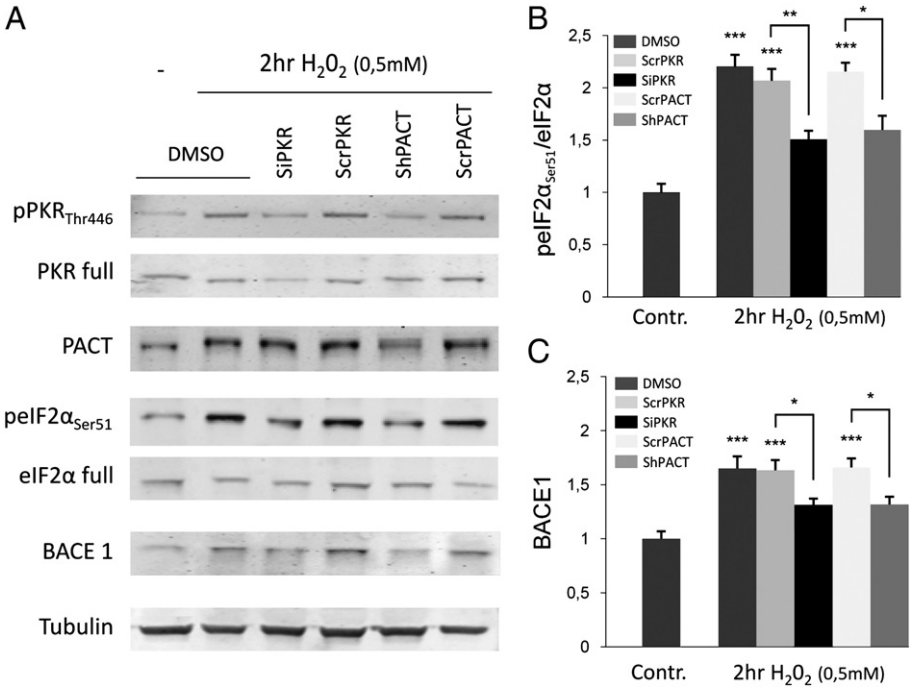

Fig. 6. Effect of H_2O_2 treatment on PKR, PACT, eIF2α, and BACE1 expression in wild type SH-SY5Y cells, or transfected with SiPKR or ShPACT. A. Western blot of SH-SY5Y proteins revealed by different antibodies specific for $pPKR_{Thr446}$, PKR full, PACT, $peIF2\alpha_{Ser51}$, eIF2α full, BACE1 and Tubulin. B–C. Relative levels of $peIF2\alpha_{Ser51}$/eIF2α ratio (B) and BACE1 (C) in SH-SY5Y transfected with SiPKR, ShPACT or their relative scramble sequence showed that PACT-PKR pathway can regulate eIF2α activation and BACE1 level. $n=3$ for each group of cells. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

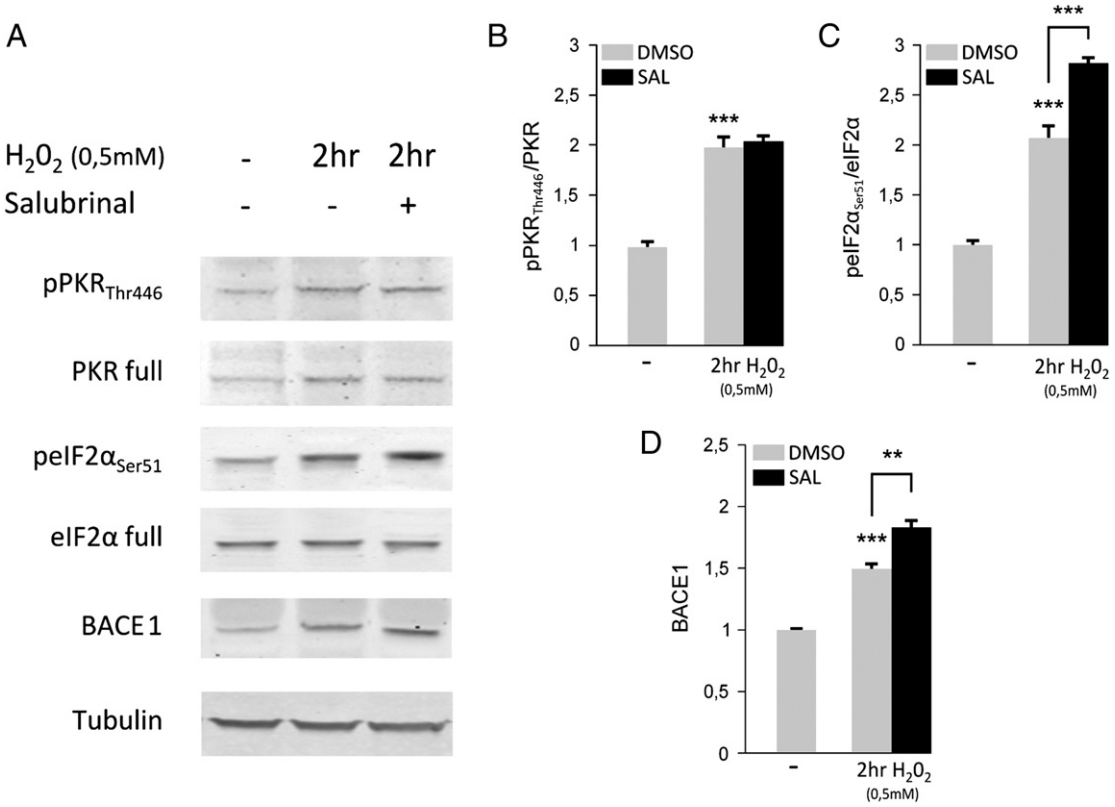

Fig. 7. Salubrinal induces significant increase of BACE1 expression in SH-SY5Y cells treated with H_2O_2. A. Western blot of SH-SY5Y cells proteins with specific antibodies for $pPKR_{Thr446}$, PKR full, $peIF2\alpha_{Ser51}$, eIF2α full, BACE1 and Tubulin. B–D. Effect of salubrinal on $pPKR_{Thr446}$/PKR ratio (B); $peIF2\alpha_{Ser51}$/eIF2α ratio (C), and BACE1 level (D) in SH-SY5Y cells treated or not with H_2O_2 (0.5 mM). All values were normalized on Tubulin level. $n=4$ for each group. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

level was significantly decreased (Fig. 8A and C). This effect was not reversed by salubrinal.

Secondly, we treated neuroblastoma cells with actinomycin D. Transcriptional inhibition decreased BACE1 level in our system (Fig. 8A and C), suggesting that a transcriptional action occurs in the control of BACE1 modulation.

To confirm the transcriptional regulation of BACE1 in this model, we conducted quantitative RT-PCR on *BACE1* mRNA. We found that *BACE1* messages were increased during OS (Fig. 8D). One hour of H_2O_2 treatment upregulates *BACE1* mRNA levels in SH-SY5Y. The addition of PRI did not significantly affect this increase (Fig. 8D).

Thirdly, we tested if BACE1 protein stability could be modulated by the ubiquitin–proteasomal degradation pathways. Combined treatment of the SH-SY5Y with H_2O_2 and lactacystin, a proteasome inhibitor, did not reveal any modification of activated eIF2α or BACE1 levels (Fig. 8A and C).

4. Discussion

This study shows that the phosphorylated forms of PKR and eIF2α as well as the levels of BACE1 are significantly increased in AD cortical regions compared to control subjects as well as in APP/PS1 KI mice. Moreover in these areas, BACE1 and activated eIF2α levels are correlated, suggesting a possible signaling interaction between these proteins in degenerating brains. In addition, our results revealed that PKR activation, during OS, can regulate BACE1 protein levels in human neuroblastoma cells.

The first question to be addressed is how PKR, eIF2α and BACE1 are linked in human brains. Previous studies have shown that these proteins are expressed in human cortical neurons [54]. In addition, we demonstrated a correlation between $peIF2\alpha_{Ser51}$ and BACE levels in frontal and temporal cortex areas, and a correlation between $peIF2\alpha_{Ser51}$ and amyloid β levels in frontal cortex. The lack of correlation between activated PKR and BACE1 could be explained by three possibilities. 1) The presence of three other identified kinases, PERK, HRI and GCN2, able to independently regulate eIF2α in response to various cellular stresses [23,24]. For example, PERK, component of the unfolded protein response, plays a critical role in the ER stress response observed in AD [55,56] and could enhance eIF2α phosphorylation in AD brains. 2) The control of BACE1 levels could take place during translation but also, by miRNA, a molecular phenomenon that are not under PKR control [19,22,57]. 3) Another factor is that activated PKR is prone to induce cell degeneration [58]. BACE1 levels could be modified during this detrimental cellular process. Nevertheless, PKR could partly influence BACE1 levels and thus amyloid β production in human brains.

In patients with AD, the rate of oxidative products is related to cerebral damage, and ample human and experimental evidences support the importance of OS in the pathogenesis of this disease [44-46,48]. Our findings demonstrated that phosphorylated PKR on threonine 446 is increased in OS condition with a transient activation from 30 minutes to 4 hours under H_2O_2 exposure, leading to an enhanced $pPKR_{Thr446}$/PKR ratio (Fig. 4A–B). In SH-SY5Y cells, a progressive enhancement of $peIF2\alpha_{Ser51}$/eIF2α ratio and BACE1 levels was also detected. In parallel, $pPERK_{Thr981}$/PERK ratio remains unchanged, indicating that eIF2α activation and BACE1 increase occurs independently of PERK activation in OS. These results concerning PKR are reminiscent of those observed in AD brains and tend to validate the fact that over-activation of PKR could induce detrimental consequences in affected cells.

Moreover, increase levels of *PKR* mRNA and PKR protein were also observed in SH-SY5Y after OS exposure (Suppl. Fig. 1A-B), as previously described in Jurkat T cells [26]. The exact mechanisms

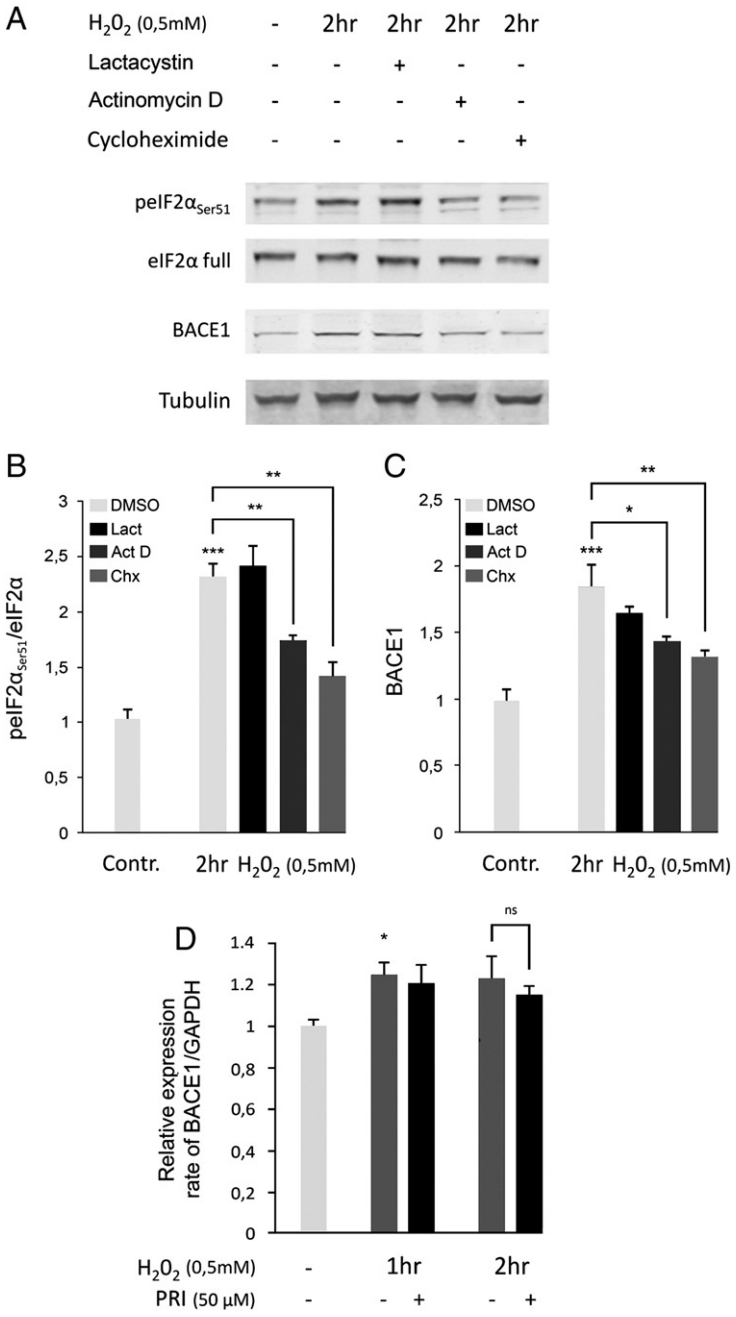

Fig. 8. Role of transcriptional, translational and proteasomal activity on BACE1 increase in OS condition. A. Effect of Actinomycin D (Act D), Cycloheximide (Chx) and Lactacystin (Lact) on BACE1 protein expression were investigated by western-blot of SH-SY5Y cells proteins with specific antibodies for $pPKR_{Thr446}$, PKR full, $peIF2\alpha_{Ser51}$, eIF2α full, BACE1 and Tubulin. B–C. Quantification of $peIF2\alpha_{Ser51}$/eIF2α ratio (B), and BACE1 level (C) in SH-SY5Y cells treated with H_2O_2 and several inhibitors. Both levels normalized on Tubulin increased with both Act D and Chx treatments, while no modification was observed after Lact exposure. Results were obtained from 3 independent experiments. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. D. Effect of OS and PRI on *BACE1* mRNA levels, assessed with qRT-PCR. *BACE1* mRNA levels were normalized to GAPDH mRNA levels, $n=4$ for each condition, ns = non significant.

triggering *PKR* expression in OS remains to be determined. However, it is already known that basal *PKR*, *PACT* and *BACE1* mRNAs expression could be modulated by OS-induced transcription factor Sp1 [59,60]. Further experimental studies by quantitative expression assays, using Sp1 inhibitors such as mithramycin will be necessary to confirm a possible transcriptional control of these transcripts under OS condition.

Our results in SH-SY5Y cells also show that PKR inhibition, carried out with pharmacological agents (PRI) or PKR siRNA, can modulate eIF2α phosphorylation and BACE1 levels (Fig. 5 and 6). The linear relation between BACE1 expression and β-secretase activity [61] suggests that amyloid β production could be also modulated in similar proportions by PKR inhibition. Modulation of eIF2α activation obtained in this study with the cell-permeable peptide PRI are comparable to previous published data in which PRI could afford neuroprotection in a similar cell culture system [53]. In addition, PKR inhibition is able to attenuate tau phosphorylation in SH-SY5Y cells, induced by Aβ or the ER stress inducer tunicamycin [35]. In the future, PKR could represent an interesting target to modulate BACE1 levels and tau phosphorylation [35,62].

Previous reports suggest that PACT may be part of the PKR activation process detected in neurons [29]. We have shown here that transfected cells with ShPACT expressed attenuated levels of both $peIF2\alpha_{Ser51}$/eIF2α ratio and BACE1, indicating that: i—PKR activation is controlled by PACT in OS condition; ii—PACT levels via PKR activation could modulate BACE1 level and activity after H_2O_2 exposure.

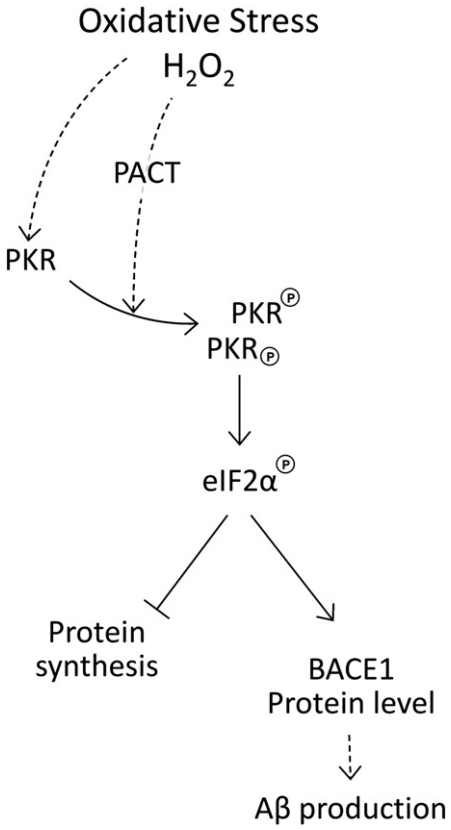

Fig. 9. Representative diagram of BACE1 regulation by PKR and eIF2α in response to oxidative stress.

Previous studies have shown that the protein PACT and *PACT* mRNA expression could be enhanced in AD brains or by various cell stresses, such as ER stress [29,63,64].

Our findings also indicated that BACE1 is regulated by a translational mechanism initiated by the PACT/PKR pathway (Fig. 7). The recent literature provides strong evidences that translation, in particular the initiation step, is a highly regulated process that plays a critical role in the cellular response to stress, and in neurodegeneration. Phosphorylation of eIF2α at Ser51 is a major mechanism for regulation of translation initiation in response to various cellular stresses [55,65]. This phosphorylation usually suppresses general translation [66], but selectively stimulates the translation of stress response transcript, that contain in their 5' UTR region, inhibitory uORFs [40,67]. A very recent study also confirms this potential role of PKR on BACE1. Activation of PKR by herpes simplex virus type 1 or dsRNA analog poly (I:C) causes Aß accumulation via derepression of BACE1 translation [68].

However, if we have clearly indentified a translational pathway that implicates PKR/eIF2α in OS, the exact role of the different BACE1 uORFs in this regulation needs to be further evaluated [65,69].

Actinomycin D treatment in SH-SY5Y cells has revealed that, during OS, a transcriptional control is also implicated in the regulation of BACE1 levels. RT-PCR analyses of BACE1 expression also confirmed the transcriptional part in BACE1 modulation by OS (Fig. 8). This is consistent with several recent studies showing that JNK and extracellular signal regulated MAP kinase (ERK) could modulate BACE1 transcription and promote amyloid β production during experimental OS [49,70,71]. Several works have shown that PKR acts upstream of JNK and may control its activation in cells treated by viruses [72,73] or other stresses (ribotoxic stress and DNA damage response) [74,75].

Taking together, our findings provide a new inter-related transcriptional and translational regulation of BACE1 expression, under the control of PKR. We can suggest here, as already proposed for the DNA damage response [74], a model of bipartite BACE1 protein expression in response to oxidative stress including activation of PACT-PKR pathway and eIF2α translational control and as well as a transcriptional regulation mediated by c-jun terminal kinase (Fig. 9). Further studies will be needed to evaluate *in vitro* the role of PKR in Aβ production.

Competing interests statement

The authors declare that they have no competing interests.

Authors' contributions

JH, FML conceived the study and drafted the manuscript. FML carried biochemical biology molecular studies. CB, FG provided human brain samples. LP provided mouse brain samples. CP. JD analyzed data.

Abbreviations

Aβ	amyloid β
ActD	Actinomycin D
AP1	activator protein 1
APP	(Amyloid precursor protein)
AD	Alzheimer's disease
BACE1	"<beta>-site" APP cleaving enzyme
C99	99 amino acid C-terminal fragment of APP
Chx	Cycloheximide
DMSO	Dimethyl sulfoxyde
eIF2α	α subunit of eukaryotic translation initiation factor-2
ER	Endoplasmic reticulum
ERK	Extracellular signal regulated MAP kinase
GADD34	Growth Arrest and DNA Damage-Inducible Protein 34
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GCN2	General control nonrepressed 2
GGA3	Golgi-associated, gamma adaptin ear containing, ARF binding protein 3
GSK-3β	Glycogen synthase kinase 3β
HRI	Heme regulated inhibitor
JNK	c-jun N-terminal kinase
Lact	lactacystin
MEM	Minimum essential media
miRNA	microRNA
OS	Oxidative stress
PACT	PKR ACTivating protein
PBS	Phosphate-buffered saline
PERK	PKR-like endoplasmic reticulum kinase
PKR	Double-stranded RNA-dependent protein kinase
PMI	Post-mortem interval
PP1	Protein phosphatase 1
PRI	Peptide PKR inhibitor
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RAX	PKR-associated protein X
RIPA buffer	Radio immune precipitation assay buffer
Sal	Salubrinal
ScrPKR	Scrambled PKR siRNA
SDS	Sodium dodecyl sulfate
ShRNA	Short hairpin RNA
SiRNA	Silencing RNA
Tau	Tubulin associated unit
uORFs	upstream open reading frames

Supplementary materials related to this article can be found online at doi:10.1016/j.bbadis.2012.01.009.

Acknowledgments

This work was supported by INSERM and APHP. The authors thank Mariko Taga, Sarah Gourmaud, Pauline Lapalus and Pierre Mazot for technical help and Eliane Meurs for kindly providing the PKR siRNA.

References

[1] C. Duyckaerts, B. Delatour, M.C. Potier, Classification and basic pathology of Alzheimer disease, Acta Neuropathol. 118 (2009) 5–36.
[2] V.W. Chow, M.P. Mattson, P.C. Wong, M. Gleichmann, An overview of APP processing enzymes and products, Neuromolecular Med. 12 (2009) 1–12.
[3] B.D. Bennett, P. Denis, M. Haniu, D.B. Teplow, S. Kahn, J.C. Louis, M. Citron, R. Vassar, A furin-like convertase mediates propeptide cleavage of BACE, the Alzheimer's beta-secretase, J. Biol. Chem. 275 (2000) 37712–37717.
[4] R. Vassar, B.D. Bennett, S. Babu-Khan, S. Kahn, E.A. Mendiaz, P. Denis, D.B. Teplow, S. Ross, P. Amarante, R. Loeloff, Y. Luo, S. Fisher, J. Fuller, S. Edenson, J. Lile, M.A. Jarosinski, A.L. Biere, E. Curran, T. Burgess, J.C. Louis, F. Collins, J. Treanor, G. Rogers, M. Citron, Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE, Science 286 (1999) 735–741.
[5] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[6] H. Cai, Y. Wang, D. McCarthy, H. Wen, D.R. Borchelt, D.L. Price, P.C. Wong, BACE1 is the major beta-secretase for generation of Abeta peptides by neurons, Nat. Neurosci. 4 (2001) 233–234.
[7] Y. Luo, B. Bolon, S. Kahn, B.D. Bennett, S. Babu-Khan, P. Denis, W. Fan, H. Kha, J. Zhang, Y. Gong, L. Martin, J.C. Louis, Q. Yan, W.G. Richards, M. Citron, R. Vassar, Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation, Nat. Neurosci. 4 (2001) 231–232.
[8] H. Fukumoto, B.S. Cheung, B.T. Hyman, M.C. Irizarry, Beta-secretase protein and activity are increased in the neocortex in Alzheimer disease, Arch. Neurol. 59 (2002) 1381–1389.
[9] L.B. Yang, K. Lindholm, R. Yan, M. Citron, W. Xia, X.L. Yang, T. Beach, L. Sue, P. Wong, D. Price, R. Li, Y. Shen, Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease, Nat. Med. 9 (2003) 3–4.
[10] J. Zhao, Y. Fu, M. Yasvoina, P. Shao, B. Hitt, T. O'Connor, S. Logan, E. Maus, M. Citron, R. Berry, L. Binder, R. Vassar, Beta-site amyloid precursor protein cleaving enzyme 1 levels become elevated in neurons around amyloid plaques: implications for Alzheimer's disease pathogenesis, J. Neurosci. 27 (2007) 3639–3649.
[11] R.M. Holsinger, C.A. McLean, K. Beyreuther, C.L. Masters, G. Evin, Increased expression of the amyloid precursor beta-secretase in Alzheimer's disease, Ann. Neurol. 51 (2002) 783–786.
[12] J. Charlwood, C. Dingwall, R. Matico, I. Hussain, K. Johanson, S. Moore, D.J. Powell, J.M. Skehel, S. Ratcliffe, B. Clarke, J. Trill, S. Sweitzer, P. Camilleri, Characterization of the glycosylation profiles of Alzheimer's beta-secretase protein Asp-2 expressed in a variety of cell lines, J. Biol. Chem. 276 (2001) 16739–16748.
[13] M.A. Christensen, W. Zhou, H. Qing, A. Lehman, S. Philipsen, W. Song, Transcriptional regulation of BACE1, the beta-amyloid precursor protein beta-secretase, by Sp1, Mol. Cell. Biol. 24 (2004) 865–874.
[14] J. Wan, A.K. Fu, F.C. Ip, H.K. Ng, J. Hugon, G. Page, J.H. Wang, K.O. Lai, Z. Wu, N.Y. Ip, Tyk2/STAT3 signaling mediates beta-amyloid-induced neuronal cell death: implications in Alzheimer's disease, J. Neurosci. 30 (2011) 6873–6881.
[15] Y. Wen, W.H. Yu, B. Maloney, J. Bailey, J. Ma, I. Marie, T. Maurin, L. Wang, H. Figueroa, M. Herman, P. Krishnamurthy, L. Liu, E. Planel, L.F. Lau, D.K. Lahiri, K. Duff, Transcriptional regulation of beta-secretase by p25/cdk5 leads to enhanced amyloidogenic processing, Neuron 57 (2008) 680–690.
[16] P. Preece, D.J. Virley, M. Costandi, R. Coombes, S.J. Moss, A.W. Mudge, E. Jazin, N.J. Cairns, Beta-secretase (BACE) and GSK-3 mRNA levels in Alzheimer's disease, Brain Res. Mol. Brain Res. 116 (2003) 155–158.
[17] D.T. Coulson, N. Beyer, J.G. Quinn, S. Brockbank, J. Hellemans, G. Brent, R. Ravid, J.A. Johnston, BACE1 mRNA expression in Alzheimer's disease postmortem brain tissue, J. Alzheimers Dis. 22 (2010) 1111–1122.
[18] Q. Li, T.C. Sudhof, Cleavage of amyloid-beta precursor protein and amyloid-beta precursor-like protein by BACE 1, J. Biol. Chem. 279 (2004) 10542–10550.
[19] S.S. Hebert, K. Horre, L. Nicolai, A.S. Papadopoulou, W. Mandemakers, A.N. Silahtaroglu, S. Kauppinen, A. Delacourte, B. De Strooper, Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 6415–6420.
[20] F. Mouton-Liger, C. Paquet, J. Hugon, Biogenesis and regulation of microRNA: implication in Alzheimer's disease, Futur. Neurol. 5 (2010) 839–850.
[21] T. Sarajarvi, A. Haapasalo, J. Viswanathan, P. Makinen, M. Laitinen, H. Soininen, M. Hiltunen, Down-regulation of seladin-1 increases BACE1 levels and activity through enhanced GGA3 depletion during apoptosis, J. Biol. Chem. 284 (2009) 34433–34443.
[22] G. Tesco, Y.H. Koh, E.L. Kang, A.N. Cameron, S. Das, M. Sena-Esteves, M. Hiltunen, S.H. Yang, Z. Zhong, Y. Shen, J.W. Simpkins, R.E. Tanzi, Depletion of GGA3 stabilizes BACE and enhances beta-secretase activity, Neuron 54 (2007) 721–737.
[23] T.E. Dever, Gene-specific regulation by general translation factors, Cell 108 (2002) 545–556.
[24] C.G. Proud, eIF2 and the control of cell physiology, Semin. Cell Dev. Biol. 16 (2005) 3–12.
[25] S.D. Der, Y.L. Yang, C. Weissmann, B.R. Williams, A double-stranded RNA-activated protein kinase-dependent pathway mediating stress-induced apoptosis, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 3279–3283.
[26] C.W. Pyo, S.H. Lee, S.Y. Choi, Oxidative stress induces PKR-dependent apoptosis via IFN-gamma activation signaling in Jurkat T cells, Biochem. Biophys. Res. Commun. 377 (2008) 1001–1006.
[27] T. Ito, M. Yang, W.S. May, RAX, a cellular activator for double-stranded RNA-dependent protein kinase during stress signaling, J. Biol. Chem. 274 (1999) 15427–15432.
[28] X. Wang, Z. Fan, B. Wang, J. Luo, Z.J. Ke, Activation of double-stranded RNA-activated protein kinase by mild impairment of oxidative metabolism in neurons, J. Neurochem. 103 (2007) 2380–2390.
[29] C. Paquet, F. Mouton-Liger, C. Bouras, C. Duyckaerts, M. Vigny, F. Gray, E. Meurs, J. Hugon, Involvement of the PKR activator PACT in Alzheimer's disease, Brain Pathol. (2012), doi:10.1111/j.1750-3639.2011.00520.x.
[30] C.V. Patel, I. Handy, T. Goldsmith, R.C. Patel, PACT, a stress-modulated cellular activator of interferon-induced double-stranded RNA-activated protein kinase, PKR, J. Biol. Chem. 275 (2000) 37993–37998.
[31] R.C. Chang, K.C. Suen, C.H. Ma, W. Elyaman, H.K. Ng, J. Hugon, Involvement of double-stranded RNA-dependent protein kinase and phosphorylation of eukaryotic initiation factor-2alpha in neuronal degeneration, J. Neurochem. 83 (2002) 1215–1225.
[32] A.L. Peel, D.E. Bredesen, Activation of the cell stress kinase PKR in Alzheimer's disease and human amyloid precursor protein transgenic mice, Neurobiol. Dis. 14 (2003) 52–62.
[33] R.C. Chang, A.K. Wong, H.K. Ng, J. Hugon, Phosphorylation of eukaryotic initiation factor-2alpha (eIF2alpha) is associated with neuronal degeneration in Alzheimer's disease, Neuroreport 13 (2002) 2429–2432.
[34] J. Hugon, C. Paquet, R.C. Chang, Could PKR inhibition modulate human neurodegeneration? Expert. Rev. Neurother. 9 (2009) 1455–1457.
[35] A. Bose, F. Mouton-Liger, C. Paquet, P. Mazot, M. Vigny, F. Gray, J. Hugon, Modulation of tau phosphorylation by the kinase PKR: implications in Alzheimer's disease, Brain Pathol. 21 (2011) 189–200.
[36] T. O'Connor, K.R. Sadleir, E. Maus, R.A. Velliquette, J. Zhao, S.L. Cole, W.A. Eimer, B. Hitt, L.A. Bembinster, S. Lammich, S.F. Lichtenthaler, S.S. Hebert, B. De Strooper, C. Haass, D.A. Bennett, R. Vassar, Phosphorylation of the translation initiation factor eIF2alpha increases BACE1 levels and promotes amyloidogenesis, Neuron 60 (2008) 988–1009.
[37] L. Devi, M. Ohno, Phospho-eIF2alpha level is important for determining abilities of BACE1 reduction to rescue cholinergic neurodegeneration and memory defects in 5XFAD mice, PLoS One 5 (2010) e12974.
[38] J. Lee, E.H. Park, G. Couture, I. Harvey, P. Garneau, J. Pelletier, An upstream open reading frame impedes translation of the huntingtin gene, Nucleic Acids Res. 30 (2002) 5110–5119.
[39] H.P. Harding, Y. Zhang, D. Ron, Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase, Nature 397 (1999) 271–274.
[40] K.M. Vattem, R.C. Wek, Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 11269–11274.
[41] D. De Pietri Tonelli, M. Mihailovich, A. Di Cesare, F. Codazzi, F. Grohovaz, D. Zacchetti, Translational regulation of BACE-1 expression in neuronal and non-neuronal cells, Nucleic Acids Res. 32 (2004) 1808–1817.
[42] S. Lammich, S. Schobel, A.K. Zimmer, S.F. Lichtenthaler, C. Haass, Expression of the Alzheimer protease BACE1 is suppressed via its 5'-untranslated region, EMBO Rep. 5 (2004) 620–625.
[43] M. Mihailovich, R. Thermann, F. Grohovaz, M.W. Hentze, D. Zacchetti, Complex translational regulation of BACE1 involves upstream AUGs and stimulatory elements within the 5' untranslated region, Nucleic Acids Res. 35 (2007) 2975–2985.
[44] B.I. Giasson, H. Ischiropoulos, V.M. Lee, J.Q. Trojanowski, The relationship between oxidative/nitrative stress and pathological inclusions in Alzheimer's and Parkinson's diseases, Free Radic. Biol. Med. 32 (2002) 1264–1275.
[45] G. Perry, M.A. Taddeo, A. Nunomura, X. Zhu, T. Zenteno-Savin, K.L. Drew, S. Shimohama, J. Avila, R.J. Castellani, M.A. Smith, Comparative biology and pathology of oxidative stress in Alzheimer and other neurodegenerative diseases: beyond damage and response, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 133 (2002) 507–513.
[46] P.J. Ebenezer, A.M. Weidner, H. LeVine III, W.R. Markesbery, M.P. Murphy, L. Zhang, K. Dasuri, S.O. Fernandez-Kim, A.J. Bruce-Keller, E. Gavilan, J.N. Keller, Neuron specific toxicity of oligomeric amyloid-beta: role for JUN-kinase and oxidative stress, J. Alzheimers Dis. 22 (2011) 839–848.
[47] P.H. Reddy, Amyloid precursor protein-mediated free radicals and oxidative damage: implications for the development and progression of Alzheimer's disease, J. Neurochem. 96 (2006) 1–13.
[48] R. Borghi, S. Patriarca, N. Traverso, A. Piccini, D. Storace, A. Garuti, C. Gabriella, O. Patrizio, T. Massimo, The increased activity of BACE1 correlates with oxidative stress in Alzheimer's disease, Neurobiol. Aging 28 (2007) 1009–1014.
[49] E. Tamagno, M. Guglielmotto, M. Aragno, R. Borghi, R. Autelli, L. Giliberto, G. Muraca, O. Danni, X. Zhu, M.A. Smith, G. Perry, D.G. Jo, M.P. Mattson, M. Tabaton, Oxidative stress activates a positive feedback between the gamma- and beta-secretase cleavages of the beta-amyloid precursor protein, J. Neurochem. 104 (2008) 683–695.

[50] C. Casas, N. Sergeant, J.M. Itier, V. Blanchard, O. Wirths, N. van der Kolk, V. Vingtdeux, E. van de Steeg, G. Ret, T. Canton, H. Drobecq, A. Clark, B. Bonici, A. Delacourte, J. Benavides, C. Schmitz, G. Tremp, T.A. Bayer, P. Benoit, L. Pradier, Massive CA1/2 neuronal loss with intraneuronal and N-terminal truncated Abeta42 accumulation in a novel Alzheimer transgenic model, Am. J. Pathol. 165 (2004) 1289–1300.
[51] M. Boyce, K.F. Bryant, C. Jousse, K. Long, H.P. Harding, D. Scheuner, R.J. Kaufman, D. Ma, D.M. Coen, D. Ron, J. Yuan, A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress, Science 307 (2005) 935–939.
[52] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Res. 29 (2001) e45.
[53] G. Page, A. Rioux Bilan, S. Ingrand, C. Lafay-Chebassier, S. Pain, M.C. Perault Pochat, C. Bouras, T. Bayer, J. Hugon, Activated double-stranded RNA-dependent protein kinase and neuronal death in models of Alzheimer's disease, Neuroscience 139 (2006) 1343–1354.
[54] E.B. Lee, B. Zhang, K. Liu, E.A. Greenbaum, R.W. Doms, J.Q. Trojanowski, V.M. Lee, BACE overexpression alters the subcellular processing of APP and inhibits Abeta deposition in vivo, J. Cell Biol. 168 (2005) 291–302.
[55] M.J. Clemens, Initiation factor eIF2 alpha phosphorylation in stress responses and apoptosis, Prog. Mol. Subcell. Biol. 27 (2001) 57–89.
[56] C.G. Proud, Regulation of eukaryotic initiation factor eIF2B, Prog. Mol. Subcell. Biol. 26 (2001) 95–114.
[57] L. Wang, H. Shim, C. Xie, H. Cai, Activation of protein kinase C modulates BACE1-mediated beta-secretase activity, Neurobiol. Aging 29 (2008) 357–367.
[58] M.A. Garcia, E.F. Meurs, M. Esteban, The dsRNA protein kinase PKR: virus and cell control, Biochimie 89 (2007) 799–811.
[59] S. Das, S.V. Ward, R.S. Tacke, G. Suske, C.E. Samuel, Activation of the RNA-dependent protein kinase PKR promoter in the absence of interferon is dependent upon Sp proteins, J. Biol. Chem. 281 (2006) 3244–3253.
[60] L.C. Platanias, Mechanisms of type-I- and type-II-interferon-mediated signalling, Nat. Rev. Immunol. 5 (2005) 375–386.
[61] Y. Li, W. Zhou, Y. Tong, G. He, W. Song, Control of APP processing and Abeta generation level by BACE1 enzymatic activity and transcription, FASEB J. 20 (2006) 285–292.
[62] S. Ingrand, L. Barrier, C. Lafay-Chebassier, B. Fauconneau, G. Page, J. Hugon, The oxindole/imidazole derivative C16 reduces in vivo brain PKR activation, FEBS Lett. 581 (2007) 4473–4478.
[63] A. Daher, G. Laraki, M. Singh, C.E. Melendez-Pena, S. Bannwarth, A.H. Peters, E.F. Meurs, R.E. Braun, R.C. Patel, A. Gatignol, TRBP control of PACT-induced phosphorylation of protein kinase R is reversed by stress, Mol. Cell. Biol. 29 (2009) 254–265.
[64] Y. Lee, I. Hur, S.Y. Park, Y.K. Kim, M.R. Suh, V.N. Kim, The role of PACT in the RNA silencing pathway, EMBO J. 25 (2006) 522–532.
[65] M. Schroder, R.J. Kaufman, Divergent roles of IRE1alpha and PERK in the unfolded protein response, Curr. Mol. Med. 6 (2006) 5–36.
[66] A.G. Hinnebusch, The eIF-2 alpha kinases: regulators of protein synthesis in starvation and stress, Semin. Cell Biol. 5 (1994) 417–426.
[67] P.D. Lu, H.P. Harding, D. Ron, Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response, J. Cell Biol. 167 (2004) 27–33.
[68] G. Ill-Raga, E. Palomer, M.A. Wozniak, E. Ramos-Fernandez, M. Bosch-Morato, M. Tajes, F.X. Guix, J.J. Galan, J. Clarimon, C. Antunez, L.M. Real, M. Boada, R.F. Itzhaki, C. Fandos, F.J. Munoz, Activation of PKR causes amyloid ss-peptide accumulation via de-repression of BACE1 expression, PLoS One 6 (2011) e21456.
[69] G.D. Pavitt, eIF2B, a mediator of general and gene-specific translational control, Biochem. Soc. Trans. 33 (2005) 1487–1492.
[70] R. Quiroz-Baez, E. Rojas, C. Arias, Oxidative stress promotes JNK-dependent amyloidogenic processing of normally expressed human APP by differential modification of alpha-, beta- and gamma-secretase expression, Neurochem. Int. 55 (2009) 662–670.
[71] Y.D. Kwak, R. Wang, J.J. Li, Y.W. Zhang, H. Xu, F.F. Liao, Differential regulation of BACE1 expression by oxidative and nitrosative signals, Mol. Neurodegener. 6 (2011) 17.
[72] K.C. Goh, M.J. deVeer, B.R. Williams, The protein kinase PKR is required for p38 MAPK activation and the innate immune response to bacterial endotoxin, EMBO J. 19 (2000) 4292–4297.
[73] M.S. Iordanov, J.M. Paranjape, A. Zhou, J. Wong, B.R. Williams, E.F. Meurs, R.H. Silverman, B.E. Magun, Activation of p38 mitogen-activated protein kinase and c-Jun NH(2)-terminal kinase by double-stranded RNA and encephalomyocarditis virus: involvement of RNase L, protein kinase R, and alternative pathways, Mol. Cell. Biol. 20 (2000) 617–627.
[74] P. Peidis, A.I. Papadakis, H. Muaddi, S. Richard, A.E. Koromilas, Doxorubicin bypasses the cytoprotective effects of eIF2alpha phosphorylation and promotes PKR-mediated cell death, Cell Death Differ. 18 (2010) 145–154.
[75] H.R. Zhou, A.S. Lau, J.J. Pestka, Role of double-stranded RNA-activated protein kinase R (PKR) in deoxynivalenol-induced ribotoxic stress response, Toxicol. Sci. 74 (2003) 335–344.