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ORIGINAL ARTICLE

β-Amyloid 1-42 induces physiological transcriptional regulation of BACE1

Alessandra Piccini,* Roberta Borghi,* Michela Guglielmotto,†,‡ Elena Tamagno,†,‡ Gabriella Cirmena,* Anna Garuti,* Valeria Pollero,§ Sergio Cammarata,§ Michele Fornaro,¶ Massimo Messa,** Laura Colombo,** Mario Salmona,** George Perry†† and Massimo Tabaton*

*Department of Internal Medicine and Medical Specialities, University of Genova, Genova, Italy

†Department of Experimental Medicine and Oncology, General Pathology Section, University of Torino, Torino, Italy

‡Neuroscience Institute of the Cavalieri Ottolenghi Foundation (NICO), University of Torino, Orbassano (Torino), Italy

§Department of Neurology, Galliera Hospital, Genova, Italy

¶Department of Neurosciences, Ophtalmology and Genetics, University of Genova, Genova, Italy

**Department of Molecular Biochemistry and Pharmacology, Mario Negri Research Institute, Milan, Italy

††College of Sciences, University of Texas at San Antonio, San Antonio, Texas, USA

Abstract

The pathogenesis of Alzheimer's disease (AD) is only partially understood. β-amyloid (Aβ) is physiologically generated by sequential cleavage of its precursor protein by the β- and the γ-secretase and it is normally disposed of. In Alzheimer's disease, Aβ is excessively produced or less dismissed, but the hypothesis on its physiological and pathological role are heterogeneous and often discordant. It has been described a positive feedback loop from the γ- to the β-secretase cleavages of Aβ precursor protein, which is activated by mutations of Presenilin 1 (PS1), the catalytic core of the γ-secretase. These findings show that Aβ precursor protein as well the activity of the γ-secretase are required to obtain the up-regulation of β-secretase which is induced by Presenilin 1 mutations. Then, Aβ 1-42 is the Aβ precursor protein derivative that up-regulates the expression of β-secretase, and c-jun N-terminal kinase (JNK)/c-Jun and ERK1/2 are involved. Here, we describe the activation of β-secretase and c-jun N-terminal kinase related proteins by monomeric Aβ 1-42, defining the conditions that most efficiently strike the described signaling without producing toxicity. Taken together these data imply that monomeric Aβ 1-42, at non-toxic concentrations and time frames, are able to induce a signaling pathway that leads to transcriptional activation of β-secretase.

Keywords: β-amyloid, β-secretase, γ-secretase, aggregation, Alzheimer's disease, Presenilins.

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The major pathologic event of Alzheimer's disease (AD) is the accumulation in the brain of β-amyloid (Aβ), heterogeneous peptide that result from the sequential cleavages operated on the β-amyloid precursor protein (APP) by the β-secretase (BACE 1) and the γ-secretase (Selkoe 2001). The altered activity of both endo-proteases is involved in the pathogenesis of AD. Presenilin 1 (PS1) is the catalytic subunit of the γ-secretase. Mutations of PS1 gene, that are

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Address correspondence and reprint requests to Massimo Tabaton, Department of Internal Medicine and Medical Specialities, University of Genova, Viale Benedetto XV 6, 16132 Genova, Italy.
E-mail: mtabaton@neurologia.unige.it

Abbreviations used: Aβ, β-amyloid; AD, Alzheimer's disease; APP, β-amyloid precursor protein; BACE 1, β-secretase; JNK, c-jun N-terminal kinase; PS1, Presenilin 1.

the major cause of early-onset familial AD, induce an altered γ-secretase cleavage, leading to a relative increased production of Aβ 42 species (Citron *et al.* 1997). The activity of BACE1 is augmented in the brain of sporadic AD (Holsinger *et al.* 2002; Yang *et al.* 2003). Oxidative stress (Tamagno *et al.* 2002) and hypoxia (Sun *et al.* 2006) are causes of the increased BACE1 activity. We, and other research groups, have shown that oxidant agents and 4-hydroxynonenal produce *in vitro* an over-expression of BACE1 (Tamagno *et al.* 2002; Kao *et al.* 2004). Then, we have demonstrated that the increase in oxidative products is significantly correlated with the increase in BACE1 activity in cerebral cortex of sporadic AD (Borghi *et al.* 2007). Studying the mechanism of the up-regulation of BACE1 induced by oxidative stress we discovered that the activation of BACE1 is regulated by the activity of the γ-secretase, and it requires an APP derivative that results from the γ-secretase cleavage (Tamagno *et al.* 2008). Furthermore, we have shown that oxidative stress induces the up-regulation of BACE1 expression via the activation of the c-jun N-terminal kinase (JNK) pathway, whereas ERK opposes this effect (Tamagno *et al.* 2009). Our findings show that a positive feed-back loop from the γ-secretase to the β-secretase cleavages of APP is induced not only by oxidative stress but also by PS1 mutations, and that Aβ 1-42 is the product of the β-secretase cleavage that up-regulates BACE1 expression (Giliberto *et al.* 2009). The effect of Aβ 1-42 on BACE1 gene transcription is mediated by the JNK/c-jun signaling pathway (Guglielmotto *et al.* 2011). Moreover, it has been determined an Aβ interacting domain in the promoters of AD-associated genes, suggesting a novel Aβ activity as a putative transcription factor (Maloney and Lahiri 2011). This suggests that a signaling pathway may be active, starting from Aβ 1-42 and ending on the BACE1 promoter. BACE1 promoter is a very complex one, and the expression of BACE1 is likely to be tightly regulated (Sambamurti *et al.* 2004).

Aβ is the subject and object of pathways leading to cell death or survival, where it could play a role not just as a toxic compound, but as a functional signaling intermediate. The hypothesis of an Aβ physiological and pathological role could rely on its effects at different concentrations, aggregation states, and timing of exposure. Amyloidogenic peptides produce solutions with heterogeneity of molecular species, responsible for affecting the reproducibility of experiments. Manzoni *et al.* (2009) have recently established a procedure to obtain repeatable physiochemical and biological features of Aβ peptides.

Aggregated Aβ leads to neuronal damage by inducing oxidative stress (Chauhan and Chauhan 2006), impairing membrane integrity (Kirkitadze and Kowalska 2005), and triggering cellular signaling leading to apoptosis (Di Carlo 2010). But Aβ both increases or impairs synaptic activity (Kamenetz *et al.* 2003; Abramov *et al.* 2009), and this paradox is likely depending from different composition, aggregation state, and time of exposure of the molecule.

Then, different Aβ peptides, full length or truncated at the N- or C-terminals, at low concentrations and for short time frames, interact with and activate several membrane receptors, e.g. TrkA and p75NTR (Bulbarelli *et al.* 2009), APP itself (Verdier *et al.* 2004), RAGE (Yan *et al.* 2009), insulin receptor (Townsend *et al.* 2007), acetylcholine receptors (Parri and Dineley 2010), leading to signaling cascades that involve the MAPK pathway, NFKB, and more, possibly controlling gene expression. This signaling action could represent a physiological role of Aβ and/or subtly contribute to the pathogenesis of AD long before the formation of plaques.

Materials and methods

Monomer and oligomer characterization

Aβ 1-42 (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGL MVGGVVIA) was prepared by solid phase peptide synthesis (SPPS) on a 433A synthesizer (Applied Biosystems, Foster City, CA, USA) using Fmoc-protected L-amino acid derivatives, NOV-ASYN-TGA resin, and a 0.1 mM scale. After the synthesis, the peptide was treated as previously described to obtained monomers and oligomers (Manzoni *et al.* 2009). This approach allows to obtain batches peptide content at constant of monomers or monomers and oligomers of low-molecular weight.

Atomic force microscopy (AFM) was carried out on a Multimode AFM with a Nanoscope V system operating in Tapping Mode using standard phosphorus-doped silicon probes (Veeco/Digital Instruments, Mannheim, Germany). The scan rate was tuned proportionally to the area scanned and was kept in the 0.5–1.2 Hz range. Peptide samples were diluted to 5 μM with 10 mM PB, and 60 μL aliquots were immediately spotted onto a freshly cleaved muscovite mica disk and incubated for 0.5 min. The disk was then washed with H_2O and dried under a gentle nitrogen stream. All the topographic patterns described in the text were confirmed by additional measurements in a minimum of five different, well-separated areas. The data processing of AFM images were handled using Scanning Probe Image Processor (SPIP-version-5.1.6 released 13 April 2011) data analysis package (Nanoscience Instruments, Phoenix, AZ, USA).

Circular dichroism (CD)

Monomers and oligomers peptide solutions were diluted to give a final concentration of 25 μM with 50 mM phosphate buffered, 150 mM NaCl, pH 7.4. The CD spectra were collected on a Jasco J-815 spectropolarimeter (Jasco, Easton, MD, USA) at 4°C from 190 to 260 nm (1.0 nm band width, and 0.1 nm resolution) using a 0.1 cm path length quartz cell. Generally, a sensitivity of 100 mdeg, a response of 16 s, a scan speed of 20 nm/min, and four accumulations were used. CD spectra were expressed as mean molar ellipticity.

Transmission electron microscopy (TEM)

Ten microliters of monomers or oligomers solutions was dropped onto nickel formvar-carbon coated 200 mesh electron microscopy grids (Electron Microscopy Science, Hatfield, PA, USA) and after 5 min the solution was removed. Samples were stained for 5 min with a saturated solution of uranyl acetate, as described previously (Gobbi *et al. 2006*). Electron microscopy analyses were done with a Libra 120 transmission electron microscope (Carl Zeiss SMT,

Gottingen, Germany) operating at 120 kV equipped with a Proscan Slow Scan CCD camera (Carl Zeiss SMT).

Cell culture and transfection

SH-SY5Y cells were cultured in RPMI 1640 (Euroclone, Milan, Italy) supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, 1% non-essential amino acids, and 1% sodium pyruvate. Mouse embryonic fibroblast (MEFs) wt and JNK1/2dko (provided by Roger Davis, University of Massachusetts, Worcester, MA, USA) were cultured in low glucose Dulbecco's modified Eagle's medium containing 2 mM glutamine and supplemented with 100 mL/L fetal bovine serum, 10 mL/L non-essential amino acids, and 10 mL/L antibiotic mixture (penicillin–streptomycin–amphotericin), in a humidified atmosphere at 37°C with 5% CO_2. Cells were transiently transfected with empty vector (pcDNA3), wild type JNK, and a dominant negative of JNK (negative control) using Lipofectamine 2000 (Life Technologies, Grand Island, NY, USA) according to the manufacturer's instructions.

Treatment of cultured cells with Aβ peptides

To obtain reproducible and certain Aβ conformations, we will use a preparation procedure that consists on the sequential use of trifluoroacetic acid, formic acid, and sodium hydroxide solutions that disaggregate preformed seeds and enriched Aβ peptide solutions into monomers and/or low-molecular-weight oligomers (Manzoni *et al.* 2009). The cells were plated the day before the experiments in completed medium; then, 30 min before the treatment with Aβ peptides, serum-free medium was added to the cells. Treatments of SH-SY5Y with Aβ peptides (1-40 monomers, 1-42 monomers, and 1-42 oligomers) were started 16–18 h after plating, aliquotes of lyophilized Aβ (monomers or oligomers) were kept at −80°C and dissolved at the time of the experiment as described (Manzoni *et al.* 2009). MEFs transiently transfected as described above were treated under the same conditions as for SH-SY5Y, 24 h after transfection. For RNA analysis cells were harvested after 20, 40, 60 min of treatment, for protein analysis cells were harvested after 1-2-3-6 or 12 h of treatment.

Western blot analysis

Whole-cell extracts were prepared in ice-cold lysing buffer [1 mL of phosphate-buffered saline was added with 10 μL Triton X100; 10 μL SDS 10%, 5 μL DTT 1 M, 6 μL PMSF 0.1%, 10 μL aprotinin] for 20 min. The lysates were cleared by centrifugation at 14 000*g* for 25 min. Nuclear cell fractions were obtained as previously described by the method of Andrew and Faller (1991). Lysates and nuclear fraction extracts were loaded on 9% Tris-Glycine SDS-PAGE gels and transferred onto PVDF membranes (Amersham Biosciences, Buckingamshire, UK). Non-specific binding was blocked with 50 g/L non-fat dry milk in 50 mM Tris-HCl, pH 7.4, containing 200 mM NaCl and 0.5 mM Tween-20 (Tris-buffered saline Tween). Blots were probed with different primary antibodies, followed by incubation with peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulins in Tris-buffered saline Tween containing 20 g/L non-fat dry milk and the reactive bands were revealed with ECL plus (Amersham Biosciences). To normalize protein levels membranes were stripped with Restore western blot stripping reagent (Pierce, Rockford, IL, USA) and probed with a monoclonal antibody against β-actin (1 : 6000; Sigma, St Louis, MO, USA). The following antibodies were used: polyclonal anti-BACE1 (Chemicon, Temecula, CA, USA); anti-pASK, anti-ASK, anti-pJNKK, anti-JNKK, anti-pJNK, anti-JNK, anti-pc-jun, anti-jun, anti–pERK, and anti-ERK antibodies (Cell Signalling Technology, Beverly, MA, USA).

Quantitative real time PCR analysis

Total RNA was extracted from 6 wells of cultured cells using the TRIZOL method, according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). Three micrograms of total RNA was reverse transcribed using random primers. Primers and probes for BACE1 were designed with the aid of Primer Express software (Applied Biosystems). To avoid amplification of contaminating genomic DNA, primers and probes were located on adjacent exons. Set primers and probe for BACE1: forward 5′-TGG AGG GCT TCT ACG TTG TCT T-3′, reverse 5′-GCT GCC GTC CTG AAC TCA TC-3′, probe 6fam-5′-TTG GCT TTG CTG TCA GCG CTT GC-3′ Tamra. Primers and probes for beta-actin were obtained from a pre-developed assay-on-demand (Applied Biosystems). Five microliters of the resulting cDNA dilution was used for quantitative PCR amplification performed, in duplicate, on the Prism 7900HT Instrument (Applied Biosystems) using the fluorescent TaqMan method. The BACE1 mRNA quantities were normalized to the control gene and were expressed in relation to a calibrator sample. The levels of transcripts BACE1 and β-actin in each sample were determined using the standard curve. The standard curve was obtained with serial dilutions (10^6–10 molecules) of the calibrator, control plasmids containing cloned sequences of ABL gene (Ipsogen, Marseille, France).

MTT assay and ROS determination

For MTT and ROS assays, SH-SY5Y cells were plated and cultured as described above and treated with Aβ species, in a 96-well plate format. Cells were then washed and incubated with MTT solution for 3 h in incubator (1/10 in culture medium serum free; stock: 5 mg/mL in PBS; Sigma Chemical Company). Solubilizing solution (10% SDS in 0.01N HCl) was then added (same volume as MTT medium) and incubated over night at 37°C, on a shaker. The next day wells were read at 570 nm with background subtraction at 620 nm, using a Benchmark Microplate Reader, with Microplate Manager Version 5.1 Software (Bio-Rad, Hercules, CA, USA).

Intracellular generation of ROS was detected by detecting the conversion of 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), internalized by cells and de-acetylated by esterase, into the corresponding fluorescent derivative. Cells were then washed and incubated with 1 μL of DCFH-DA 1 mM for 30 min at 37°C (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 100 μL serum-free culture medium without phenol red. After treatment, cells were washed and lysed in 100 μL DMSO 90% and fluorescence was measured on a Fluostar Optima microplate reader (BMG Labtechnologies, Offenburg, Germany; excitation 485 nm, emission 520 nm).

RNA interference

RNA interference to knockdown the JNK1/2 in MEFs cells were performed using a RNAi human/mouse control kit, including the small RNA duplex (Qiagen, Milan, Italy). The siRNA (2.5 μg) were transfected into cells using the proper RNAiFect reagent ratio (1 : 6)

in medium for 48 h. Transfected cells in fresh medium were then exposed to 100 nM Aβ1-42 for up to 12 h and then harvested for sample preparation.

Statistical analysis

Data in bar graphs represent mean ± SD and were obtained from average data of at least three independent experiments. Luminograms images are representative of at least three experiments with similar results. Statistical analysis was performed with GraphPad (GraphPad Software Inc., La Jolla, CA, USA), using Student's *t*-test or ANOVA test followed by the Bonferroni post-test when appropriate ($p < 0.05$ was considered significant).

Results

In a previous article (Giliberto *et al.* 2009), we showed how BACE1 is transcriptionally regulated by soluble Aβ 1-42. As reports indicate that Aβ oligomers may have a signaling action, we conducted the same experiments using oligomeric preparations of Aβ compared with monomeric forms (see Figure S1 for a detailed description of different peptide assemblies). We did not used fibrils, as the main effect of these preparations is toxicity. To obtain reproducible and certain Aβ conformations, we used a preparation procedure that consists on the sequential use of trifluoroacetic acid, formic acid, and sodium hydroxide solutions that disaggregate preformed seeds and enriched Aβ peptide solutions into monomers and/or low-molecular-weight oligomers (Manzoni *et al.* 2009). We used monomeric or oligomeric forms of Aβ at the lowest possible concentration and for the shortest timeframe: we treated SHSY-5Y cells with 100 nM to 1 μM concentrations, for 20–40 min up to 1–2 h. Monomeric Aβ 1-42 incubation induced BACE1 mRNA level starting from 20 min incubation and became statistically significant at 1 h (*$p < 0.05$ for Aβ 100 nM, ***$p < 0.001$ for Aβ 1 μM; Fig. 1a). This event is paralleled by an augmented protein synthesis induced by monomeric Aβ 1-42 as shown by western blot analysis of BACE1 protein (*$p < 0.05$ for Aβ 100 nM, **$p < 0.01$ for Aβ 1 μM; Fig. 1b). Thus, we confirm the activation of BACE1 by Aβ 1-42 at different aggregations states and time points. As control, BACE1 mRNA and protein levels are not affected by treatment with monomeric Aβ 1-40 (Fig. 2).

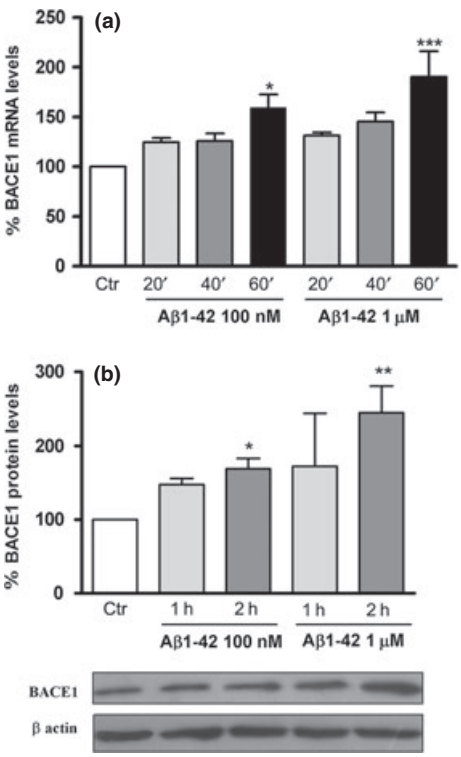

Fig. 1 Effect of monomeric Aβ 1-42 on β-secretase (BACE1) levels on SH-SY5Y neuroblastoma cells. (a) BACE1 mRNA levels increase from 20 min of incubation with monomeric Aβ 1-42 and the difference become statistically significant at 1 h (*$p < 0.05$ for Aβ 100 nM, ***$p < 0.001$ for Aβ 1 μM). (b) BACE1 protein levels increase from 1 h of incubation with monomeric Aβ 1-42 and the difference become statistically significant at 2 h (*$p < 0.05$ for Aβ 100 nM, **$p < 0.01$ for Aβ 1 μM).

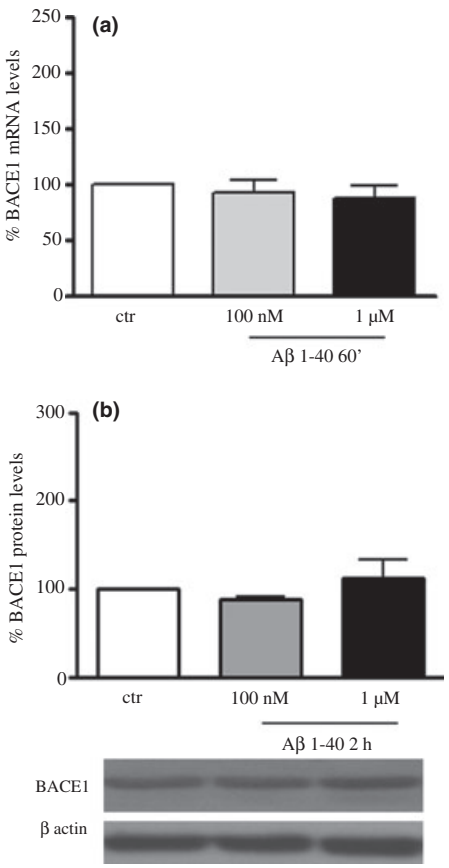

Fig. 2 Effect of monomeric Aβ 1-40 on β-secretase (BACE1) levels on SH-SY5Y neuroblastoma cells. BACE1 mRNA (a) and protein (b) levels do not change after treatment with monomeric Aβ 1-40 100 nM and 1 μM even at the longer time frame.

After having determined the best experimental conditions for monomeric Aβ 1-42-induced BACE1 up-regulation, we ascertained that monomeric Aβ 1-42, as employed in our model, has no toxic effect on the cell. We studied the effect of monomeric Aβ 1-42, at the different conditions tested, on SH-SY-5Y neuroblastoma cells viability (MTT assay) and oxidative stress (ROS production), as described before (Tamagno *et al.* 2008). As positive control, cells have been incubated with oligomeric Aβ 1-42 as soluble oligomers are the toxic form of the peptide, at least at the onset of the pathology (Di Carlo 2010). Monomeric Aβ 1-42 has no effect on cell viability at the different concentrations used (Fig. 3a) nor on ROS production (Fig. 4a) as compared with the oligomeric toxic form (Figs 3b and 4b). As control, cells viability and oxidative stress are not affected by treatment with monomeric Aβ 1-40 (data not shown).

To investigate the mechanism of BACE1 transcriptional regulation, we focused on the JNK signaling pathway as it can be activated by Aβ peptides (Wang *et al.* 2004; Kadowaki *et al.* 2005; Yao *et al.* 2005). MEFs wild type treated with monomeric Aβ 1-42 100 nM showed the activation of JNK and related proteins, e.g. apoptosis signal-regulating kinase 1 (ASK-1), the jun kinase kinase (JNKK), and c-jun (Fig. 5). As JNK and ERK pathways have a dual opposite effect on the expression of BACE 1 (Tamagno *et al.* 2009), we investigated the effect of Aβ 1-42 on ERK. As expected, treatment with monomeric Aβ 1-42 100 nM up to 6 h inhibits the induction of ERK (Fig. 5). Activation of ERK is restored at longer time of exposure, 12 h, when pJNK and pc-jun are down-regulated (Fig. 5).

We then treated MEFs JNK1/2dko with monomeric Aβ 1-42 100nM: basal expression levels of BACE1 are low in these cells (Fig. 6), and monomeric Aβ 1-42 did not change the expression of BACE1 compared to the significant increase induced in the wild type ($*p < 0.05$, $**p < 0.01$; Fig. 6). Remarkably, transfection with JNK wild type, but not with a JNK dominant negative construct (Fig. 7), reconstituted the effect of monomeric Aβ 1-42 on BACE1 expression, as quantified by the relative histograms ($*p < 0.05$, $**p < 0.01$). Silencing of JNK with RNAi significantly blocked the increase in BACE1 protein levels induced by monomeric Aβ 1-42 (Fig. 8), as confirmed by the densitometric analysis ($**p < 0.01$). These data showed how non-toxic monomeric Aβ 1-42 activates, in a positive feedback loop, the transcription of BACE1, with the activation of the JNK/c-jun pathway.

Discussion

Our study identifies a physiological function of monomeric Aβ 1-42 able to induce a signaling pathway that leads to

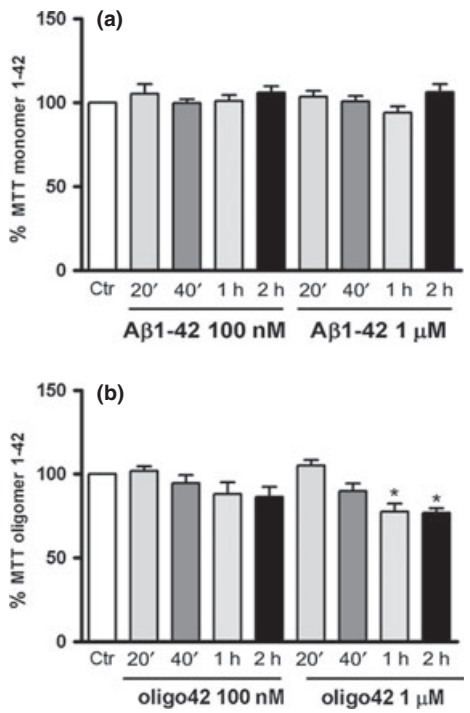

Fig. 3 Effect of Aβ 1-42 on SH-SY-5Y neuroblastoma cells viability (MTT assay). (a) Monomeric Aβ 1-42 has no effect on cell viability at the different concentrations used. (b) Oligomeric Aβ 1-42 is toxic for the cells. Cells viability decreases and the difference becomes statistically significant after 1 h of incubation with 1 μM oligomeric Aβ 1-42 ($*p < 0.05$).

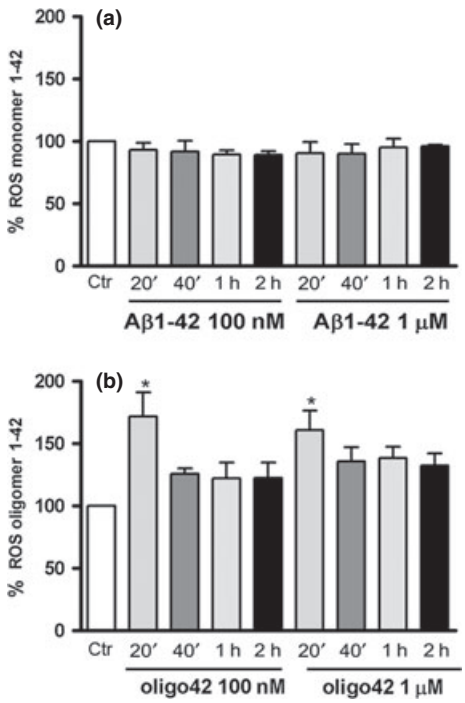

Fig. 4 Effect of Aβ 1-42 on SH-SY-5Y neuroblastoma cells oxidative stress (ROS production). (a) Monomeric Aβ 1-42 has no effect on oxidative stress at the different concentrations used. (b) Oligomeric Aβ 1-42 is toxic for the cells, oxidative stress is significantly increased ($*p < 0.05$).

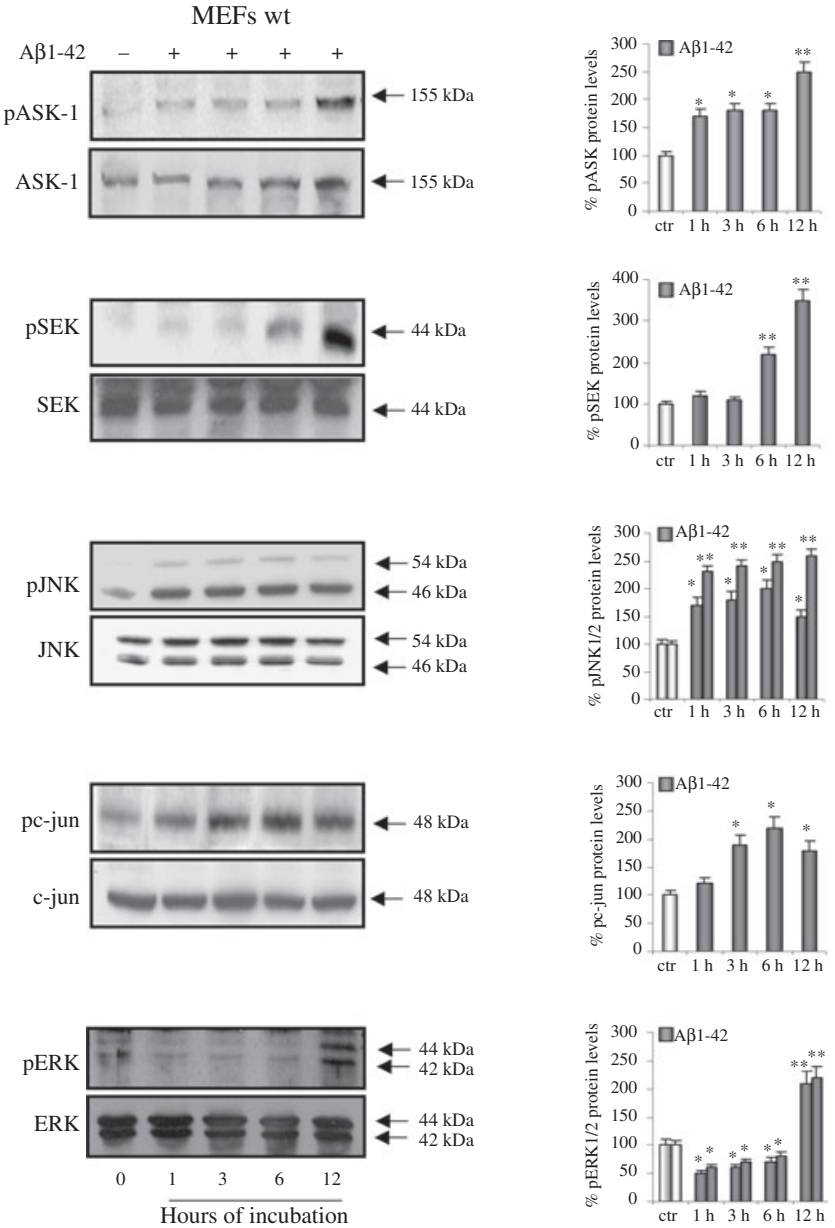

Fig. 5 Effect of monomeric Aβ 1-42 100 nM on the c-jun N-terminal kinase (JNK) signaling pathway in MEFs wild-type cells. Monomeric Aβ 1-42 100 nM induces activation of JNK and related proteins, e.g. apoptosis signal-regulating kinase 1 (ASK-1), the jun kinase kinase (JNKK), and c-jun. As expected 1–6 h treatment with monomeric Aβ 1-42 100 nM inhibits activation of ERK. $^{*}p < 0.05$; $^{**}p < 0.01$.

transcriptional activation of BACE1 without causing toxicity to the cell. Previous studies have proposed different physiological functions of Aβ (Kamenetz *et al.* 2003; Plant *et al.* 2003). Aβ and BACE1 are pivots of several signaling pathways, involving different cellular receptors and signal transduction mechanisms (Thathiah and De Strooper 2009; Tabaton *et al.* 2010), leading to heterogeneous cellular effects, from toxicity to synaptic modulation, apoptosis or cell survival (Ulrich *et al.* 1998; Diarra *et al.* 2009). Aβ production is dependent on BACE1 (Bennett *et al.* 2000), but we and others have shown how BACE1 is regulated by soluble Aβ at the transcriptional level, defining a positive control loop that fosters Aβ production (Giliberto *et al.* 2009; Jo *et al.* 2010). The specific significance of this regulation is unknown, other than perpetrating the ominous consequences of amyloid accumulation (Liu *et al.* 2002). APP similarity to NOTCH and to its processing strengthen the idea that APP and its derivatives may have a physiological signaling role. Aβ binds several ligands and receptors, but the experimental conditions in which synthetic or endogenous Aβ are employed are, again, very heterogeneous (Yaar *et al.* 2007; Lauren *et al.* 2009; Tabaton *et al.* 2010). The hypothesis on Aβ physiological role are heterogeneous and often discordant, as consensus is lacking on its effects at different concentrations, aggregation states, and time of exposure. This could be because of

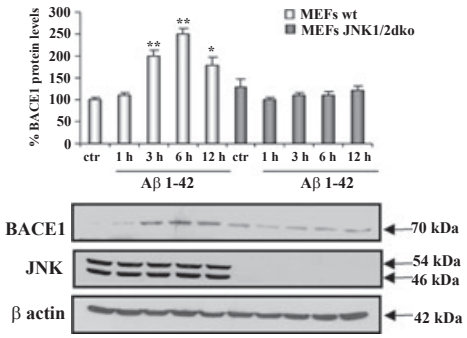

Fig. 6 β-secretase (BACE1) protein levels in MEFs wild type and in MEFs c-jun N-terminal kinase (JNK)1/2dko cells. Basal levels of BACE1 are low in MEFs c-jun N-terminal kinase 1/2dko and monomeric Aβ 1-42 did not change the levels of BACE1 compared to the significant increase induced in the wild type (*$p < 0.05$, **$p < 0.01$).

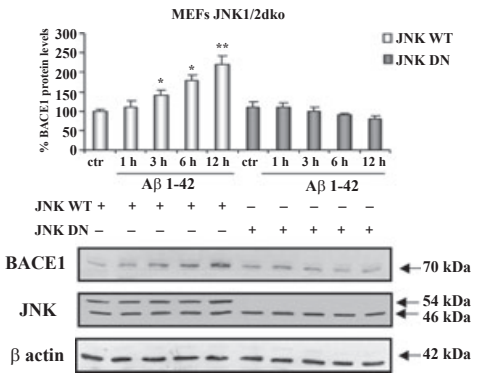

Fig. 7 Effect of c-jun N-terminal kinase (JNK) transfection on β-secretase (BACE1) expression. Transfection in MEFs JNK1/2dko with JNK wild type (WT) but not with a JNK dominant negative (DN) construct, reconstituted the effect of monomeric Aβ 1-42, as quantified by the relative histograms (*$p < 0.05$, **$p < 0.01$).

physicochemical and biological variability with different batches of amyloidogenic peptides that have been reported in literature. To avoid this crucial experimental step we used a procedure that enable us to obtain reproducible biological features of Aβ peptides, irrespective to their age (Manzoni *et al.* 2009). Using this preparation, we showed that monomeric Aβ is able to trigger BACE1 transcription without affecting cell survival suggesting that the boundary separating toxicity from a probable physiological signaling of Aβ is very narrow. Our results stressed out the importance of the aggregation-dependent biological effect of Aβ that could be dualistic as it has been reported for its role as either an oxygen radical generator or its inhibitor (Zou *et al.* 2002).

We showed an induction of BACE1 occurring at low concentrations of monomeric preparations of Aβ 1-42 and starting within minutes of treatment. Although this time may seem short, specific gene transcription can occur within

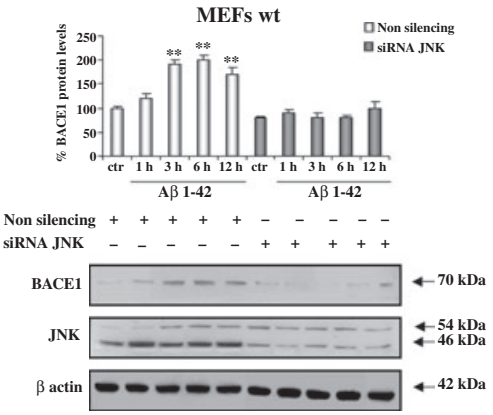

Fig. 8 Silencing of c-jun N-terminal kinase (JNK) with RNAi. The silencing in MEFs wild type of c-jun N-terminal kinase significantly blocked the increase in β-secretase (BACE1) protein levels induced by monomeric Aβ 1-42, as confirmed by the densitometric analysis (**$p < 0.01$).

minutes for certain cellular processes (Mira and Castano 1989). It is likely that BACE1 is part of a cellular response aimed at fast adaptation to extracellular stimuli. BACE1 is highly regulated, it is not only the APP β-secretase. Several secretase and non-secretase functions have been ascribed to BACE1, as the activation of voltage-gated Na channels (Huth *et al.* 2009), myelination control and processing molecules other than APP (Lichtenthaler *et al.* 2003; Wong *et al.* 2005; Willem *et al.* 2006). It is thus possible that BACE1, given its complex promoter structure and its relationships with heterogeneous groups of signaling pathways (Sambamurti *et al.* 2004; Buggia-Prevot *et al.* 2008; Tamagno *et al.* 2009), can be part of a pool of enzymes that respond to cellular stress or homeostasis modifications. As in the case of glucose lowering signals, where insulin and metformin have an opposite effect on amyloidogenesis and BACE1 regulation (Chen *et al.* 2009), in some pathways the same endpoint can results in opposite outcomes on BACE1 activation. This may be because of the fact that BACE1 is working together with still undefined partners to fulfill a cellular response.

As described previously (Guglielmotto *et al.* 2011), also monomeric Aβ 1-42 at non-toxic concentration promotes, in a positive feedback loop, the transcription of BACE1, with the activation of the JNK/c-jun pathway. Although the mechanisms of JNK activation by Aβ 1-42 is still unclear, it is likely that JNK is indirectly activated by Aβ 1-42, perhaps by the interaction of Aβ 1-42 with yet unidentified receptors. Indeed, different proteins, such as APP itself, NMDA, TrkA, and LRP family of receptors, have been shown to interact with Aβ peptides (Bu *et al.* 2006; Lauren *et al.* 2009). This could lead not only to BACE1 regulation but also to the control of a pool of genes involved in a specific cellular function. The knowledge of the genes activated by Aβ may

be determinant to understanding the precise series of events that cause dysfunction and degeneration of neurons in AD.

Our data analyze the functional significance of Aβ-induced signaling, its effect on BACE and on other independent pathways and have implications on the pathogenesis of AD, in terms of understanding these pathways all the way toward the specific cellular receptors involved. Consequently, the study may indicate strategies of therapy different from those only focused on halting the accumulation of Aβ in the brain. Furthermore, defining the network of genes in which BACE1 is called in to operate, in response to amyloidogenic stimuli, will allow to better understand its still unclear function and find ways to safely modulate its activity.

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Supporting information

Additional supporting information may be found in the online version of this article:

Figure S1. Analysis of the molecular assemblies of monomeric and oligomeric forms of Aβ 1-42.

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