Published in final edited form as:

Neurochem Int. 2008 May ; 52(6): 1030–1036. doi:10.1016/j.neuint.2007.10.020.

AMYLOID- β PEPTIDE BINDS TO MICROTUBULE-ASSOCIATED PROTEIN 1B (MAP1B)

Goar Gevorkian^{a,*}, Alfonso Gonzalez-Noriega^a, Gonzalo Acero^a, Jorge Ordoñez^a, Colette Michalak^a, Maria Elena Munguia^a, Tzipe Govezensky^a, David H. Cribbs^{b,c}, and Karen Manoutcharian^a

a Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico (UNAM), Apartado Postal 70228, Ciudad Universitaria, Mexico DF, CP 04510, MEXICO

b The Institute for Brain Aging and Dementia, University of California Irvine, Irvine, CA 92697-4540

c Department of Neurology, University of California Irvine, Irvine, CA 92697-4540

Abstract

Extracellular and intraneuronal formation of amyloid-beta aggregates have been demonstrated to be involved in the pathogenesis of Alzheimer's disease. However, the precise mechanism of amyloid-beta neurotoxicity is not completely understood. Previous studies suggest that binding of amyloid-beta to a number of targets have deleterious effects on cellular functions. In the present study we have shown for the first time that amyloid-beta 1-42 bound to a peptide comprising the microtubule binding domain of the heavy chain of microtubule-associated protein 1B by the screening of a human brain cDNA library expressed on M13 phage. This interaction may explain, in part, the loss of neuronal cytoskeletal integrity, impairment of microtubule-dependent transport and synaptic dysfunction observed previously in Alzheimer's disease.

Keywords

amyloid-beta peptide; phage displayed cDNA library; microtubule-associated protein 1B (MAP1B)

The accumulation of amyloid-beta (A β) peptide aggregates in the brain has been hypothesized to play a central role in the neuropathology of Alzheimer's disease (AD). It has been shown that extracellular and intraneuronal formation of A β deposits are involved in the pathogenesis of AD (Gouras et al., 2000; Takahashi et al., 2002; Chromy et al., 2003; Oddo et al., 2003; Fernandez-Vizarra et al., 2004; Walsh and Selkoe, 2004; Wirths et al., 2004; Aleardi et al., 2005; Billings et al., 2005; Vasilevko and Cribbs, 2006; Yang et al., 2007). However, the precise mechanism of A β neurotoxicity is not completely understood. Previous studies showed that extracellular A β interacts with a number of cell surface proteins and induces cell death as a result of an increased production of hydrogen peroxide and formation of toxic free radicals, inhibition of acetylcholine release and perturbation of Ca²⁺ homeostasis as well as pathological activation of signal transduction pathways like tau phosphorylation with subsequent

Corresponding author: Goar Gevorkian, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico (UNAM), Apartado Postal 70228, Ciudad Universitaria, Mexico DF, CP 04510, MEXICO, Tel: 5255-56223151; Fax: 56223369. Email: gokar@servidor.unam.mx.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

neurofibrillary tangle formation and increased tyrosine phosphorylation of focal adhesion kinase (Behl et al., 1994; Zhang et al., 1994; Busciglio et al., 1995; Butterfield et al., 2004; Huang et al., 2004; Kar et al., 2004; Pereira et al., 2004; Bales et al., 2006).

Intraneuronal accumulation of A β 1-42 has been studied extensively as well and it has been shown that A β interacted with tau protein and with a tau peptide containing the microtubule binding domain (Perez et al., 2004). Binding of A β to the 20 S proteasome described previously may explain the inhibition of the chymotrypsin-like activity in the proteasome and the accumulation of ubiquitin conjugates in AD (Gregori et al., 1997). Also, it has been shown that A β interacted with intracellular protein ERAB (endoplasmic reticulum amyloid β -peptide-binding protein) proved to be an L-3-hydroxyacyl-CoA dehydrogenase type II (NADH2) (Yan et al., 1997; He et al., 1998). In addition, others have proposed that this interaction may promote mitochondrial dysfunction and cell death and have suggested that inhibition of ERAB (also known as ABAD) - A β interaction may provide a new treatment strategy against AD (Lustbader et al., 2004; Takuma et al., 2005; Yan et al., 2005). Other examples of deleterious cellular events caused by intracellular interactions of A β are the inhibition of cytochrome oxidase, α -ketoglutarate dehydrogenase and pyruvate dehydrogenase activities demonstrated in isolated brain mitochondria, suggesting that A β can directly disrupt mitochondrial function (Casley et al., 2002; Canevari et al., 2004; Yan and Stern, 2005). Recently, we identified another mitochondrial enzyme, ND3 of the human A β -related death-inducing protein (AB-DIP) has been identified and shown to be involved in neuronal apoptosis (Lakshmana et al., 2005).

In the present study we have shown that A β 1-42 bound to a peptide comprising the microtubule binding domain of the heavy chain of microtubule-associated protein 1B (MAP1B) by screening a human brain cDNA library expressed on M13 phage. This interaction may explain, in part, the loss of neuronal cytoskeletal integrity, impairment of microtubule-dependent transport and synaptic dysfunction observed previously in AD (Pike et al., 1992; Busciglio et al., 1995; Praprotnik et al., 1996; Kokubo et al., 2005; Michaelis et al., 2005; Butler et al., 2007). To our knowledge, the present study is the first demonstration of binding of A β to MAP1B.

2. MATERIALS AND METHODS

2.1. Materials

Restriction enzymes, DNA isolation/purification kits, DNA polymerase, T4 DNA ligase and helper phage were obtained from Amersham (NJ, USA), Invitrogen (CA, USA), or Qiagen (CA, USA). The oligonucleotides were synthesized at Invitrogen. A β 1-42 and biotinylated A β 1-42 were obtained from AnaSpec, CA, USA. Polyclonal rabbit anti-MAP1B antibody H-130 and goat polyclonal anti-MAP1B antibody N-19 were provided by Santa Cruz Biotechnology, CA, USA. Mouse monoclonal anti-human β -amyloid antibody 4G8 was obtained from Sigma, USA. AlexaFluor 594 anti-rabbit, AlexaFluor 594 anti-goat and AlexaFluor 488 anti-mouse antibodies were provided by Molecular Probes, OR, USA.

2.2. Construction of phage display cDNA library

Construction of M13 phage display human brain cDNA library was carried out essentially as described in our previous study (Munguia et al., 2006). All molecular biology procedures were carried out using standard protocols (Sambrook et al., 1989) or as recommended by manufacturers. As a cloning vector pCANTAB-5E (Amersham) phagemid vector, permitting the expression of foreign polypeptides as fusions with the M13 phage minor coat protein (pIII), was used. As a source of cDNA, a T7 Select Human Alzheimer's Brain cDNA library (Novagen, Germany) was used. The T7 bacteriophages from this library were used as a template in a PCR with two primers, 5SFT7:

TCATATGCTCGGCCAGCCGGCCATGCTCGGGGATCCGAATTC and 3BT7: AATCTTAGTCTAGATCTTTACTCGAGTGCGGCCGCAAGCTT, carrying *Sfi* I and *Not* I restriction sites (underlined), respectively. The PCR products were gel purified, digested with *Sfi* I and *Not* I, and column purified. About 1 µg of this DNA was ligated to approximately 0.5 µg of *Sfi* I/*Not* I digested and gel-purified pCANTAB-5E vector DNA using T4 DNA ligase. The ligated DNA was column purified and introduced into *Escherichia coli* TG1 cells by electroporation using Gene Pulser II System (Bio-Rad Laboratories, CA, USA). Ten electroporations were performed, and the transformed TG1 cells were plated on LB-Amp plates to determine the diversity of the library. Ten individual bacterial colonies were used to analyze the quality of the library by PCR. The resultant phagemid library was rescued/amplified using M13KO7 helper phage, purified by double PEG/NaCl (20% w/v polyethylene glycol-8000; 2.5 M NaCl) precipitation and resuspended in Tris-buffered saline (TBS). The typical phage yields were 10^{10} – 10^{11} colony-forming units (cfu) per milliliter of culture medium.

2.3. Biopanning

To identify peptides/proteins that bind to Aβ1-42, a biopanning using the constructed phage-displayed library was carried out essentially as described previously (Munguia et al., 2006). First, an aliquot of the constructed library was incubated overnight at 4°C with biotinylated Aβ1-42 (AnaSpec) diluted in PBS, then this solution was added to the Reacti-Bind streptavidin coated plate (Pierce, IL, USA) and plate was incubated 2 hr at 37°C. After incubation, the plate was washed with cold PBS-Tween and bound phage particles were eluted using glycine-HCl (0.2 M, pH 2.2) and neutralized by adding Tris-HCl (1 M, pH 9.1). The eluted phages were plated on LB-Amp plates, and individual colonies from the second round of panning were rescued/amplified using M13KO7 helper phage and used in ELISA screening as described (Munguia et al., 2006).

2.4. DNA sequencing

The DNA sequences of the inserts of three positive clones were determined using automated ABI Prism 310 Genetic Analyzer (Applied Biosystems, CA, USA), miniprep-purified double-stranded DNA from phagemid clones and pCANTAB-5E vector-based 5' and 3' primers. The DNA and deduced amino acid sequences were analyzed by computer search with ExPASy Molecular Biology server and BLAST database.

2.5. ELISA

To analyze the binding of Aβ1-42 to selected phage (designated C8), an ELISA assay using amplified and purified phage was carried out as previously described (Manoutcharian et al., 2004; Munguia et al., 2006). Nunc maxisorp microtiter plates (Nunc, Denmark) were coated overnight with Aβ1-42 (AnaSpec) at a concentration of 2 µg/ml in carbonate buffer (pH 9.6). A non-related peptide used as a negative control (NRP; amino acid sequence: AALSPGSSAYPSATVLA) was synthesized in our laboratory. After washing with phosphate buffer containing 0.2% Tween-20 (PBS-Tween), plates were blocked with PBS/non-fat milk (2%) for 1 h at room temperature. Plates were washed, then phage (C8 and a control non-related phage) previously incubated for 30 minutes at room temperature with PBS/milk/Triton, were added at a concentration of 10^{11} per ml, and after incubation for 2 hrs at room temperature, plates were washed with PBS-Tween. HRP/Anti-M13 monoclonal conjugate (Amersham) diluted 1:5000 in PBS/2% non-fat milk/0.2% triton was added, and plates were incubated for 1 h at room temperature followed by washing step and incubation with ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) single solution (Zymed laboratories Inc., CA, USA). OD readings at 405 nm were registered using Opsys MR Microplate Reader (DYNEX Technologies, VA, USA).

2.6. Western Blot

Phage preparation was analyzed by gel electrophoresis and Western Blot. 10^{11} phage particles diluted in 16 μ l of loading buffer were boiled 3 minutes and separated on 4–12% NuPAGE Bis-Tris gel (Invitrogen) at 200 V for 45 min at room temperature as recommended by manufacturer and detected by conventional silver staining. For Western Blot analysis, peptides were electrophoretically transferred onto a nitrocellulose membrane (Invitrogen) and the membranes were incubated for 1 h at room temperature in PBS containing 2% non-fat dry milk and 0.2% Triton X-100 (PBS/milk/triton) to eliminate non-specific binding followed by overnight incubation at 4°C with anti-M13 pIII monoclonal antibody (New England Biolabs, MA, USA) or HRP-conjugated anti-E-tag antibody (Amersham) diluted 1:1000 in PBS/milk/triton. The membranes incubated with anti-M13 pIII antibody were washed several times and then incubated for 2 hrs at room temperature in PBS/milk/triton containing the HRP-conjugated anti-mouse IgG2a secondary antibody (Zymed) at a dilution of 1:2000. Immunoreactive bands were detected using 3,3'-diaminobenzidine (Sigma, MO, USA).

2.7. Immunocytochemistry

SH-SY5Y cells obtained from American Type Culture Collection (ATCC, VA, USA) were plated on cover slips and differentiated in the presence of retinoic acid for 7 days. Differentiated cells were washed with PBS and fixed for 30 min at room temperature with 4% paraformaldehyde in PBS. Then cells were incubated in 15 mM NH_4Cl for 5 min, permeabilized with 0.25% Triton X-100 for 30 min and blocked for 30 min with PBS/3% BSA. For fluorescent labeling cells were incubated 1 h at room temperature with primary antibody diluted in PBS/BSA. Polyclonal rabbit anti-MAP1B antibody H-130, goat polyclonal anti-MAP1B antibody N-19 and mouse monoclonal anti-human β -amyloid antibody 4G8 were used as primary antibodies at the following dilutions: H-130 1:2000; N-19 1:2000; 4G8 1:1000. After rinsing, cells were incubated for 1 h with AlexaFluor 594 anti-rabbit (1:4000), AlexaFluor 594 anti-goat (1:4000) or AlexaFluor 488 anti-mouse (1:1000) antibodies. After washing with PBS, cells were mounted onto glass slides in ProLong Gold antifade reagent with DAPI (Molecular Probes) and viewed and photographed with an OLYMPUS DP70 Microscope equipped for epifluorescence with a Fluotar $\times 100$ objective.

3. RESULTS

To identify peptides/proteins that bind to A β 1-42, we screened a human brain cDNA library expressed on M13 phage. After two rounds of biopanning using A β as a target, 3 positive clones were obtained. These clones were demonstrated to bear the same peptide insert comprising the basic region of the heavy chain of MAP1B containing the KKEE and KKEVI motifs (aa 594-735) and known to bind to microtubules. The phage clone expressing the fragment of MAP1B was designated C8.

To confirm the expression of the recombinant fusion protein (MAP1B-pIII), we performed WB analysis of phage C8 (Fig. 1). Wild type M13 phage was used as a control. As shown on Fig. 1, anti-pIII antibody recognizes two bands in C8 phage: one corresponding to pIII and one corresponding to the larger fusion protein MAP1B-pIII. Only one band corresponding to pIII is observed for wild type M13 phage. On the other hand, anti-E-tag antibody directed against the E-Tag peptide present in pCANTAB-5E phagemid vector, recognizes the only band in C8 phage corresponding to MAP1B-pIII fusion protein.

Binding of A β 1-42 to phage C8 was analyzed by ELISA (Fig. 2). C8 binds selectively to A β 1-42 but not to a non-related peptide. No binding to A β 1-42 was observed when control wild-type phage was used.

To confirm that A β binds the microtubule binding domain of the heavy chain of MAP1B that is localized at the amino terminal part of the protein, we decided to test first if A β 1-42 and MAP1B colocalize in cultured SH-SY5Y human neuroblastoma cells. Differentiated SH-SY5Y cells were incubated in the presence of mouse anti-A β 17-28 antibody (4G8) and goat polyclonal anti-human MAP1B antibody (N-19) raised against the N-terminus of MAP1B heavy chain (MAP1B-HC). As shown in Fig 3, both proteins are distributed in cell bodies and processes. High magnification of merged image shows that whereas A β was in small particles (300–600 nm) that could represent intracellular vesicles, amino terminal fragments of MAP1B were surrounding these small vesicles. Double immunofluorescence staining confirmed that A β and amino terminal fragment of MAP1B colocalized mainly at perinuclear regions and proximal side of processes. The specificity of this interaction was corroborated by double staining of the cells with polyclonal rabbit antibodies directed against the carboxy end of human MAP1B, MAP1B-LC. As shown in Fig 3 G and H in merged images, no interaction between these two proteins can be detected.

4. DISCUSSION

Numerous studies have demonstrated that A β accumulates intracellularly after either endogenous production or uptake of extracellular A β (Oddo et al., 2003; Walsh, et al., 2000; Fernandez-Vizarra, 2004; Billings et al., 2005; LaFerla et al., 2007). It has been suggested that intraneuronal A β accumulation was an early pathological step in AD neuropathology (Gouras et al., 2000). Also, it has been demonstrated that A β oligomers ultrastructurally localized to cell processes: they were found on presynaptic active zone and postsynaptic dendrites (Kokubo et al., 2005). Authors proposed that this accumulation of A β might be related to synaptic alterations shown to be an early event in the pathogenesis of AD (Hamos et al., 1989; Iversen et al., 1995; Walsh and Selkoe, 2004). However, the precise mechanism whereby intracellular A β aggregates disrupt the normal functioning of neurons remains to be elucidated. The search for A β -binding partners using combinatorial approaches may help to find some pieces comprising the puzzle of A β -induced cell damage.

In the present study we performed the screening against A β 1-42 of a human brain cDNA library expressed on M13 phage and found that A β 1-42 binds in ELISA to a phage clone bearing a peptide comprising the basic region of the heavy chain of MAP1B (MAP1B-HC) containing the KKEE and KKEVI motifs (aa 594-735) and known to bind to microtubules. In addition, we demonstrated that in SH-SY5Y cells MAP1B co-localizes with A β . It has been shown that A β is produced in intracellular cholesterol-rich compartments and then transported to the plasma membrane (Morishima-Kawashima et al., 1998; Mizuno et al., 1999). Also, it has been demonstrated that oligomerization of A β begins intracellularly and that about 80 % of A β oligomers are localized within processes (Walsh et al., 2000; Kokubo et al., 2005). Finally, accumulation of A β aggregates was found in the cytoplasm of neurons (Gouras et al., 2000; Buckig et al., 2002). In the present study we demonstrated that A β 1-42 and MAP1B colocalized in differentiated SH-SY5Y human neuroblastoma cells. The colocalization was observed with anti-MAP1B-HC antibodies but not with anti-MAP1B carboxy terminal (or light chain) antibodies confirming that A β 1-42 binds specifically to the N-terminal region of MAP1B. Our observations may look like somewhat contradictory since it is difficult to explain how MAP1B may bind to a peptide localized at the lumen of vesicles. We may hypothesize that MAP1B-HC interacts with A β aggregates or monomers adsorbed to vesicle membranes.

MAP 1B is a neural specific microtubule-associated protein that is the first MAP expressed by neurons in situ and present in axons, somata and dendrites (Tucker et al., 1989; Riederer 2007). MAP1B is involved in a variety of cell functions. It promotes microtubule assembly and is essential for differentiation and growth of neuronal processes as well as for the maintenance of LTP (Takemura R et al, 1992; Gonzalez-Billault, 2004; Zervas et al., 2005;

Riederer 2007). In addition, MAP1B was identified to be a membrane glycoprotein and was localized as an integral membrane protein in vesicles and the plasma membrane of neurons (Tanner et al., 2000; Franzen et al., 2001). These findings suggest that MAP1B may play a role in interactions between microtubules and surface membranes and in interactions between the neuronal cytoskeleton and components of the extracellular matrix or surface membrane of adjacent cells as well as modulate glia-axon interactions by binding to myelin-associated glycoprotein (Tanner et al., 2000; Franzen et al., 2001; Riederer 2007). Finally, it has been demonstrated that MAP1B may bind a variety of proteins (the heavy polypeptide of myosin, several heat shock proteins, alpha synuclein, HLA DR associated protein I, MAP1A, gigaxonin, the glutamate receptor-interacting protein 1 (GRIP1), the rho1 subunit of the GABA receptor, the spectrin beta chain among others) either directly, or via tubulin and actin suggesting that MAP1B may play a role as a scaffold protein by linking a number of cell molecules to the cytoskeleton (Billups et al, 2000; Seog 2004; Cueille et al., 2007; Riederer et al., 2007). Collectively, these observations suggest that MAP1B may play an important role in development and function of the nervous system, and interfering with its' functions may disrupt essential cell processes that involve this protein (Edelmann et al., 1996).

The integrity of microtubules, a major component of the cytoskeleton, is essential for neurons to maintain their morphology and to transport cell components between cell body and synaptic terminals, and MAP1B together with tau, MAP1A and MAP2 play an important role to promote tubulin assembly and to stabilize microtubules. Impairment of microtubule-dependent transport and presence of axonopathies have been reported in different animal models of AD suggesting that these phenomena may underlie cognitive decline observed in patients (Avila et al., 1992; Praprotnik et al., 1996; Kins and Beyreuther, 2006). Our findings that A β 1-42 binds to microtubule-binding domain of the MAP1B heavy chain (MAP1B-HC) may explain, in part, alterations of microtubule dynamics and structure as well as inhibition of neuronal plasticity and regeneration. Thus, it has been demonstrated that the cytoskeleton is an early cellular target for intracellular A β 1-42 aggregates and that the disruption of the microtubule network is required for A β -induced neuronal cell death (Mudher and Lovestone, 2002; Sponne et al., 2003; Butler et al., 2007). It has been shown that when exposed to soluble A β 1-42, most of the neurons displayed a disrupted microtubule architecture, even after 3 h of incubation and before the morphological and biochemical alterations typical of apoptotic cell death (Sponne et al., 2003). In addition, in that study authors also demonstrated that the perturbations of the microtubules precede caspase activation and nuclear DNA fragmentation and condensation. Finally, it has been proposed that MAP1B may be involved in cell death in neurodegenerative disorders triggered by A β deposition, although the exact molecular events remains to be understood (Uchida, 2002).

Although our findings do not explain all components of the puzzle regarding how A β causes neuronal damage, they do provide another potentially important pathogenic mechanism that may contribute to the onset and progression of AD. A better understanding of the biochemical events leading to AD will probably open a route for the discovery of new treatment strategies by interfering in interaction between A β and MAP1B.

Acknowledgements

This work was supported by grant from DGAPA-UNAM (IN203706) to K.M. and by grants from NIH: AG 023534 to G.G. and AG-20241 and NS-050895 to D.H.C. J.O. is a fellow from CONACyT and DGAPA-UNAM, MEXICO.

References

- Aleardi AM, Benard G, Augereau O, Malgat M, Talbot JC, Mazat JP, Letellier T, Dachary-Prigent J, Solaini GC, Rossignol R. Gradual alteration of mitochondrial structure and function by β -amyloids:

- importance of membrane viscosity changes, energy deprivation, reactive oxygen species production, and cytochrome c release. *J Bioenerg Biomembr* 2005;37:207–225. [PubMed: 16167177]
- Avila J, Lim F, Moreno F, Belmonte C, Cuellar AC. Tau function and dysfunction in neurons. Its role in neurodegenerative disorders. *Mol Neurobiol* 2002;25:213–231. [PubMed: 12109872]
- Bales KR, Tzavara ET, Wu S, Wade MR, Byaster FP, Paul SM, Nomkots GG. Cholinergic dysfunction in a mouse model of Alzheimer disease is reversed by an anti-A beta antibody. *J Clin Invest* 2006;116:825–832. [PubMed: 16498501]
- Behl C, Davis JB, Lesley R, Schubert D. Hydrogen peroxide mediates amyloid beta protein toxicity. *Cell* 1994;77:817–827. [PubMed: 8004671]
- Billings LM, Oddo S, Green KN, McGaugh JL, LaFerla FM. Intraneuronal A β causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron* 2005;45:675–688. [PubMed: 15748844]
- Billups D, Hanley JG, Orme M, Attwell D, Moss SJ. GABA α receptor sensitivity is modulated by interaction with MAP1B. *J Neurosci* 2000;20:8643–8650. [PubMed: 11102469]
- Buckig A, Tikkanen R, Herzog V, Schmitz A. Cytosolic and nuclear aggregation of the amyloid β -peptide following its expression in the endoplasmic reticulum. *Histochem Cell Biol* 2002;118:353–360. [PubMed: 12432446]
- Busciglio J, Lorenzo A, Yeh J, Yankner BA. Beta-amyloid fibrils induce tau phosphorylation and loss of microtubule binding. *Neuron* 1995;14:879–888. [PubMed: 7718249]
- Butler D, Bendiske J, Michaelis ML, Karanian DA, Bahr BA. Microtubule-stabilizing agent prevents protein accumulation-induced loss of synaptic markers. *Eur J Pharmacol* 2007;562:20–27. [PubMed: 17336290]
- Butterfield DA, Boyd-Kimball D. Amyloid beta-peptide (1–42) contributes to the oxidative stress and neurodegeneration found in Alzheimer disease brain. *Brain Pathol* 2004;14:426–432. [PubMed: 15605990]
- Canevari L, Abramov AY, Duchon MR. Toxicity of amyloid β peptide: tales of calcium, mitochondria, and oxidative stress. *Neurochem Res* 2004;29:637–650. [PubMed: 15038611]
- Casley CS, Canevari L, Land JM, Clark JB, Sharpe MA. β -Amyloid inhibits integrated mitochondrial respiration and key enzyme activities. *J Neurochem* 2002;80:91–100. [PubMed: 11796747]
- Chromy BA, Nowak RJ, Lambert MP, Viola KL, Chang L, Velasco PT, Jones BW, Fernandez SJ, Lacor PN, Horowitz P, Finch CE, Krafft GA, Klein WL. Self-assembly of A β (1–42) into globular neurotoxins. *Biochemistry* 2003;42:12749–12760. [PubMed: 14596589]
- Cueille N, Tallichet Blanc C, Pota-Nita S, Kasas S, Catsicas S, Dietler G, Riederer BM. Characterization of MAP1B heavy chain interaction with actin. *Brain Res Bull* 2007;71:610–618. [PubMed: 17292804]
- Edelmann W, Zervas M, Costello P, Roback L, Fischer I, Hammarback JA, Cowan N, Davies P, Wainer B, Kuchelapati R. Neuronal abnormalities in microtubule-associated protein 1B mutant mice. *Proc Natl Acad Sci USA* 1996;93:1270–1275. [PubMed: 8577753]
- Fernandez-Vizarra P, Fernandez AP, Castro-Blanco S, Serrano J, Bentura ML, Martinez-Murillo R, Martinez A, Rodrigo J. Intra- and extracellular A β and PHF in clinically evaluated cases of Alzheimer's disease. *Histol Histopathol* 2004;19:823–844. [PubMed: 15168346]
- Franzen R, Tanner SL, Dashiell SM, Rottkamp CA, Hammer JA, Quarles RH. Microtubule-associated protein 1B: a neuronal binding partner for myelin-associated glycoprotein. *J Cell Biol* 2001;155:893–898. [PubMed: 11733546]
- Gonzalez-Billault C, Jimenez-Mateos EM, Caceres A, Diaz-Nido J, Wandosell F, Avila J. Microtubule-associated protein 1B function during normal development, regeneration, and pathological conditions in the nervous system. *J Neurobiol* 2004;58:48–59. [PubMed: 14598369]
- Gouras GK, Tsai J, Naslund J, Vincent B, Edgar M, Checler F, Greenfield JP, Haroutunian V, Buxbaum JD, Xu H, Greengard P, Relkin NR. Intraneuronal A β 42 accumulation in human brain. *Am J Pathol* 2000;156:15–20. [PubMed: 10623648]
- Gregori L, Hainfeld JF, Simon MN, Goldgaber D. Binding of amyloid beta protein to the 20 S proteasome. *J Biol Chem* 1997;272:58–62. [PubMed: 8995227]
- Hamos JE, DeGennaro LJ, Drachman DA. Synaptic loss in Alzheimer's disease and other dementias. *Neurology* 1989;39:355–361. [PubMed: 2927643]

- He XY, Schulz H, Yang SY. A human brain L-3-hydroxyacyl-coenzyme A dehydrogenase is identical to an amyloid β -peptide-binding protein involved in Alzheimer's disease. *J Biol Chem* 1998;273:10741–10746. [PubMed: 9553139]
- Huang X, Moir RD, Tanzi RE, Bush AI, Rogers JT. Redox-active metals, oxidative stress, and Alzheimer's disease pathology. *Ann NY Acad Sci* 2004;1012:153–163. [PubMed: 15105262]
- Iversen LL, Mortishire-Smith RJ, Pollack SJ, Shearman MS. The toxicity in vitro of beta-amyloid protein. *Biochem J* 1995;311:1–16. [PubMed: 7575439]
- Kar S, Slowikowski SP, Westaway D, Mount HT. Interactions between beta-amyloid and central cholinergic neurons: implications for Alzheimer's disease. *J Psychiatry Neurosci* 2004;29:427–441. [PubMed: 15644984]
- Kins S, Beyreuther K. Teasing out the tangles. *Nat Med* 2006;12:764–765. [PubMed: 16829938]
- Kokubo H, Kaye R, Glabe CG, Yamaguchi H. Soluble A β oligomers ultrastructurally localize to cell processes and might be related to synaptic dysfunction in Alzheimer's disease brain. *Brain Res* 2005;1031:222–228. [PubMed: 15649447]
- LaFerla F, Green KN, Oddo S. Intracellular amyloid- β in Alzheimer's disease. *Nat Rev Neurosci* 2007;8:499–509. [PubMed: 17551515]
- Lakshmana MK, Araki W, Tabira T. Amyloid beta peptide binds a novel death-inducing protein, AB-DIP. *FASEB J* 2005;19:1362–1364. [PubMed: 15923395]
- Lustbader JW, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, Caspersen C, Chen X, Pollak S, Chaney M, Trinchese F, Liu S, Gunn-Moore F, Lue LF, Walker DG, Kuppusamy P, Zewier ZL, Arancio O, Stern D, Yan SS, Wu H. A β AD directly links A β to mitochondrial toxicity in Alzheimer's disease. *Science* 2004;304:448–452. [PubMed: 15087549]
- Manoutcharian K, Acero G, Munguia ME, Montero JA, Govezensky T, Cao C, Ugen K, Gevorkian G. Amyloid-beta peptide specific single chain Fv antibodies isolated from an immune phage display library. *J Neuroimmunol* 2003;145:12–17. [PubMed: 14644026]
- Michaelis ML, Seyb KI, Ansar S. Cytoskeletal integrity as a drug target. *Curr Alzheimer Res* 2005;2:227–229. [PubMed: 15974922]
- Mizuno T, Nakata M, Haiki H, Michikawa M, Wang R, Haass C, Yanagisawa K. Cholesterol-dependent generation of a seeding amyloid β protein in cell culture. *J Biol Chem* 1999;274:15110–15114. [PubMed: 10329717]
- Morishima-Kawashima M, Ihara Y. The presence of amyloid β -protein in the detergent insoluble membrane compartment of human neuroblastoma cells. *Biochemistry* 1998;37:15247–15253. [PubMed: 9799484]
- Mudher A, Lovestone S. Alzheimer's disease - do tauists and Baptists finally shake hands? *Trends Neurosci* 2002;25:22–26. [PubMed: 11801334]
- Munguia ME, Govezensky T, Martinez R, Manoutcharian K, Gevorkian G. Identification of amyloid-beta 1-42 binding protein fragments by screening of a human brain cDNA library. *Neurosci Lett* 2006;397:79–82. [PubMed: 16384638]
- Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kaye R, Metherate R, Mattson MP, Akbari Y, LaFerla FM. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular A β and synaptic dysfunction. *Neuron* 2003;39:409–421. [PubMed: 12895417]
- Pike CJ, Cummings BJ, Cotman CW. β -amyloid induces neuritic dystrophy in vitro: similarities with Alzheimer pathology. *NeuroReport* 1992;3:769–772. [PubMed: 1421135]
- Pereira C, Ferreira E, Cardoso SM, de Oliveira CR. Cell degeneration induced by amyloid-beta peptides: implications for Alzheimer's disease. *J Mol Neurosci* 2004;23:97–104. [PubMed: 15126695]
- Perez M, Cuadros R, Benitez MJ, Jiménez JS. Interaction of Alzheimer's disease amyloid beta peptide fragment 25-35 with tau protein, and with a tau peptide containing the microtubule binding domain. *J Alzheimers Dis* 2004;6:461–467. [PubMed: 15505366]
- Praprotnik D, Smith MA, Richey PL, Vinters HV, Perry G. Filament heterogeneity within the dystrophic neuritis of senile plaques suggests blockage of fast axonal transport in Alzheimer's disease. *Acta Neuropathol* 1996;91:226–235. [PubMed: 8834534]
- Riederer BM. Microtubule-associated protein 1B, a growth-associated and phosphorylated scaffold protein. *Brain Res Bull* 2007;71:541–558. [PubMed: 17292797]

- Sambrook, J.; Fritsch, EF.; Maniatis, T. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY: 1989.
- Seog DH. Glutamate receptor-interacting protein 1 protein binds to the microtubule associated protein. *Biosci Biotechnol Biochem* 2004;68:1808–1810. [PubMed: 15322371]
- Sponne I, Fifre A, Drouet B, Klein C, Koziel V, Pincon-Raymond M, Olivier JL, Chambaz J, Pillot T. Apoptotic neuronal cell death induced by the non-fibrillar amyloid- β peptide proceeds through an early reactive oxygen species-dependent cytoskeleton perturbation. *J Biol Chem* 2003;278:3437–3445. [PubMed: 12435748]
- Takahashi RH, Milner TA, Li F, Nam EE, Edgar MA, Yamaguchi H, Beal MF, Xu H, Greengard P, Gouras GK. Intraneuronal Alzheimer A β 42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am J Pathol* 2002;161:1869–1879. [PubMed: 12414533]
- Takemura R, Okabe S, Umeyama T, Kanai Y, Cowan NJ, Hirokawa N. Increased microtubule stability and alpha tubulin acetylation in cells transfected with microtubule-associated proteins MAP1B, MAP2 or tau. *J Cell Sci* 1992;103:953–964. [PubMed: 1487506]
- Takuma K, Yao J, Huang J, Xu H, Chen X, Luddy J, Trillat AC, Stern DM, Arancio O, Yan SS. ABAD enhances Abeta-induced cell stress via mitochondrial dysfunction. *FASEB J* 2005;19:597–598. [PubMed: 15665036]
- Tanner S, Franzen R, Jaffe H, Quarles RH. Evidence for expression of some microtubule-associated protein 1B in neurons as a plasma membrane glycoprotein. *J Neurochem* 2000;75:553–562. [PubMed: 10899930]
- Tucker RP, Garner CC, Matus A. In situ localization of microtubule-associated protein mRNA in the developing and adult rat brain. *Neuron* 1989;2:1245–1256. [PubMed: 2624748]
- Uchida Y. Overexpression of full-length but not N-terminal truncated isoform of microtubule-associated protein (MAP) 1B accelerates apoptosis of cultured cortical neurons. *J Biol Chem* 2003;278:366–371. [PubMed: 12376528]
- Vasilevko V, Cribbs DH. Novel approaches for immunotherapeutic intervention in Alzheimer's disease. *Neurochem Int* 2006;49:113–126. [PubMed: 16765487]
- Walsh DM, Tseng BP, Rydel RE, Podlisny MB, Selkoe DJ. The oligomerization of amyloid beta-protein begins intracellularly in cells derived from human brain. *Biochemistry* 2000;39:10831–10839. [PubMed: 10978169]
- Walsh DM, Selkoe DJ. Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron* 2004;44:181–193. [PubMed: 15450169]
- Wirths O, Multhaup G, Bayer TA. A modified beta-amyloid hypothesis: intraneuronal accumulation of the beta-amyloid peptide - the first step of a fatal cascade. *J Neurochem* 2004;91:513–520. [PubMed: 15485483]
- Yan SD, Fu J, Soto C, Chen X, Zhu H, Al-Mohanna F, Collison K, Zhu A, Stern E, Saido T, Tohyama M, Ogawa S, Roher A, Stern D. An intracellular protein that binds amyloid- β peptide and mediates neurotoxicity in Alzheimer's disease. *Nature* 1997;389:689–695. [PubMed: 9338779]
- Yan SD, Stern DM. Mitochondrial dysfunction and Alzheimer's disease: role of amyloid-beta peptide alcohol dehydrogenase (ABAD). *Int J Exp Pathol* 2005;86:161–171. [PubMed: 15910550]
- Yang HQ, Ba MW, Ren RJ, Zhang YH, Ma JF, Pan J, Lu GQ, Chen SD. Mitogen activated protein kinase and protein kinase c activation mediate promotion of sAPPa secretion by deprenyl. *Neurochem Int* 2007;50:74–82. [PubMed: 16973242]
- Zervas M, Opitz T, Edelmann W, Wainer B, Kucherlapati R, Stanton PK. Impaired hippocampal long-term potentiation in microtubule-associated protein 1B-deficient mice. *J Neurosci Res* 2005;82:83–92. [PubMed: 16118800]
- Zhang C, Lambert MP, Bunch C, Barber K, Wade WS, Krafft GA, Klein WL. Focal adhesion kinase expressed by nerve cell lines shows increased tyrosine phosphorylation in response to Alzheimer's A beta peptide. *J Biol Chem* 1994;269:25247–25250. [PubMed: 7929215]

Fig. 1. PAGE and western blot analysis of recombinant phage expressing the fragment of MAP1B. 10^{11} phage particles diluted in loading buffer were resolved on 4–12% NuPAGE Bis-Tris gel (Invitrogen) at 200 V for 45 min at room temperature and immunoblotted for detection with anti-pIII and anti-E-tag antibodies. Wild-type M13 phage was used as a control. Migration of the molecular mass standards as well as pIII and MAP1B-pIII fusion protein are indicated by arrowheads. Note, that pIII band in the wild-type M13 phage has a slightly high MW compared with the pIII band in recombinant phagemid C8 due to deletions present in the cloning vector.

Fig. 2.

Analysis of interaction of Aβ1-42 with C8 phage bearing the fragment of MAP1B. Phage concentration used was 10^{11} per ml, and 100 μ l were added to each well. M13 phage and a non-related peptide (NRP) were used as negative controls. OD at 405 was registered. Data are means of three independent experiments.

Fig. 3.

Double immunofluorescence staining of A β and MAP1B in differentiated SY5Y cells.

Differentiated cells were fixed and doubly immunostained with monoclonal mouse anti-human A β 17-24 antibody (B and F) and polyclonal goat anti-human MAP1B-HC (A) or rabbit anti-human MAP1B-LC antibodies (E). The primary antibodies were visualized with AlexaFluor 594 (green) anti-rabbit, AlexaFluor 594 (green) anti-goat or AlexaFluor 488 (red) anti-mouse antibodies. When anti-MAP1B-HC antibody was used, in merged image (C) and merged image at higher magnification (D) colocalizations of A β and MAP1B appear yellow. When staining of the cells was performed with anti-human MAP1B-LC antibody, in merged images (G) and merged image at higher magnification (H) no colocalizations were observed. Scale bar - 40 μ m.