Wnt signaling pathway overcomes the disruption of neuronal differentiation of neural progenitor cells induced by oligomeric amyloid β-peptide

Adi Shruster,* Hagit Eldar-Finkelman,† Eldad Melamed* and Daniel Offen*

*The Neuroscience Laboratory, Felsenstein Medical Research Center, Petach Tikva, Tel Aviv University, Tel Aviv, Israel

†Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Abstract

Neural stem cells give rise to new hippocampal neurons throughout adulthood. Defects in neurogenesis are associated with cognitive dysfunctions, such as Alzheimer disease (AD). Our understanding of the signals controlling this process is limited. The present *in vitro* study explored the manner in which the Wnt signaling pathway regulates the differentiation of hippocampal progenitors (HPs) into neurons under the influence of amyloid β_{42} (Aβ_{42}). The results showed that oligomeric Aβ_{42} reduced neuronal differentiation. This process was accompanied by a reduction in active β-catenin levels and proneural gene expression. The addition of Wnt3a increased the neuronal differentiation of Aβ_{42}-treated HPs, at the expense of astrocyte differentiation. The effect of Wnt signaling was attributable to progenitor cell differentiation to the neuronal lineage, and not to increased proliferation or rescue of neurons. The interruption of Wnt signaling by oligomeric Aβ_{42} may have clinical implications for the treatment of impaired neurogenesis in AD.

Keywords: Alzheimer's disease, amyloid β-peptide, hippocampus, neural stem cells, neurogenesis, Wnt signaling.

J. Neurochem. (2011) **116**, 522–529.

Most cells in the adult brain are generated during the embryonic and postnatal periods. However, recent research has shown that neurogenesis occurs throughout the adult lifespan, in two distinct regions of the brain: the subventricular zone lining the lateral ventricles and the subgranular zone of the dentate gyrus in the hippocampal formation (Toni *et al.* 2008; Zhao *et al.* 2008; Jessberger and Gage 2009).

The new neurons in the hippocampus integrate into the existing circuitry and are involved in hippocampal properties. They have also been found to be relevant and even critical to several learning and memory processes (Bruel-Jungerman *et al.* 2007a,b; Deng *et al.* 2010).

Alzheimer disease (AD) is a progressive neurodegenerative disease that affects 5% of the population over age 65 years. It is characterized by the formation of insoluble inclusions of amyloid plaques and neurofibrially tangles. The degenerative cascade is apparently initiated by an accumulation of amyloid β (Aβ), especially Aβ42 (Eckman and Eckman 2007; Haass and Selkoe 2007). Clinically, AD manifests as a wide range of cognitive impairments, the most prominent and earliest being the inability to recall recent activities (Mohs *et al.* 2000; Caselli *et al.* 2006). The ability to generate new neurons throughout life diminishes with age

and AD, both conditions involving prominent cognitive impairment. The underlying mechanism for the reduced neurogenesis in AD involves the failure of newborn neurons to mature (Drapeau and Nora Abrous 2008; Waldau and Shetty 2008; Lazarov and Marr 2010; Shruster *et al.* 2010).

Wnt ligands are important extracellular factors that regulate neural stem cell proliferation and differentiation (Ming and Song 2005; Michaelidis and Lie 2008; Nusse *et al.* 2008; Toledo *et al.* 2008; Inestrosa and Arenas 2010). *In vitro* and *in vivo* studies have shown that Wnt3 is expressed in the hippocampal neurogenic niche and modulates the generation of newborn neurons in the dentate gyrus. The inhibition of Wnt signaling by lentiviral expression of a

dominant-negative Wnt in the adult dentate gyrus decreased neurogenesis in the hippocampus (Lie *et al.* 2005). Accordingly, a model of low density lipoprotein receptor-related protein 6 mutant mice with generalized defects in the Wnt/beta-catenin signaling pathway exhibited a reduced production of dentate granule neurons and abnormalities of the radial glial scaffolding in the newly forming dentate gyrus (Zhou *et al.* 2004).

In humans, Wnt signaling appears to be altered or involved in the pathophysiology of AD (Inestrosa and Arenas 2010). This finding was supported by reports of a lower renewal capacity of glial progenitor cells isolated from the cortex of patients with AD than in cells from healthy controls (He and Shen 2009). The cells from the patients showed reduced levels of β-catenin and the proneural factors Ngn2, NeuroD1, and Mash1. Treating the glial precursor cells from healthy controls with $A\beta_{42}$ reduced neurogenesis, and the transfection of β-catenin restored it.

The aim of the present experimental study was to examine the role of Wnt signaling in neurogenesis under the influence of $A\beta_{42}$. We demonstrated that treatment of embryonic hippocampal progenitors (HPs) with monomeric $A\beta_{42}$ led to a reduction in neurogenesis, active β-catenin level, and proneural gene expression. The addition of Wnt3a completely reversed these findings. The increased neuronal lineage production was because of an increased number of HPs that differentiated to neurons, and not to the proliferation or rescue of immature neurons. A similar role of Wnt signaling in patients with AD could have clinical implications.

Materials and methods

HPs cultures

Animal studies were approved by the Animal Care and Use Committee of Tel Aviv University. All experiments were performed on C57BL/6 mice (Harlan, Jerusalem, Israel).

Hippocampi were dissected on embryonic day 17. Cell suspensions were grown in a defined medium (DMF12) composed of Dulbecco's modified Eagle's medium (DMEM)/F12 (1 : 1), 2 mM L-glutamine, 100 μg/mL streptomycin, 100 U/mL penicillin, 12.5 U/mL nystatin (Biological Industries, Beit-Haemek, Israel), 0.66% glucose, 14.6 mM $NaHCO_3$, 5 mM HEPES buffer (Sigma, St. Louis, MO, USA), B27 (Invitrogen, Chicago, IL, USA), 20 ng/mL mouse embryonic growth factor (EGF), and basic fibroblast growth factor (bFGF) (Peprotech, Rocky Hill, NJ, USA). The cells grew as neurospheres and were passaged by mechanical dissociation every week.

Wnt3a conditioned medium

Wnt3a-secreting L cells and control L cells (American Type Culture Collection, Manassas, VA, USA) were cultured in DMEM containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 12.5 U/mL nystatin (Biological Industries), and 400 μg/mL G418 (Sigma). Wnt3a-conditioned medium and control L-cell-conditioned medium were prepared by culturing the cells in DMF12 without growth factors for 4 days. The media were harvested and sterilized using a 0.22-μm filter. Fresh medium was added, and the cells were cultured for another 3 days. The media were again collected and combined with the previous media.

$A\beta_{42}$ preparation

Lyophilized $A\beta_{42}$ purified by HPLC was purchased from American Peptide (Sunnyvale, CA, USA) and reconstituted in dry dimethylsulfoxide (Sigma) at a concentration of 250 μM. Monomeric, oligomeric, and fibrillar $A\beta_{42}$ were prepared as described by Dahlgren *et al.* (2002). For oligomeric conditions, DMEM/F12 was added, bringing the peptide to a final concentration of 100 μM, followed by incubation at 4°C for 24 h; for fibrillar conditions, 10 mM HCl was added, bringing the peptide to a final concentration of 100 μM, followed by incubation at 37°C for 24 h; for monomeric conditions, 250 μM of $A\beta_{42}$ in dimethylsulfoxide was diluted directly into the cell culture media.

Transmission electron microscopy

Samples (10 μL) from the three forms of $A\beta_{42}$ were placed on 400-mesh copper grids covered by a carbon-stabilized Formvar film (SPI Supplies, West Chester, PA, USA). After 1.5 min, excess fluid was removed, and the grids were negatively stained with 10 μL of 2% uranyl acetate solution for 2 min. Excess fluid was removed, and the samples were viewed under a JEM-1200EX electron microscope (Joel, Tokyo, Japan) operating at 80 kV.

Treatment of HPs

After three passages, cells were plated into coated tissue culture plates at a density of 100 000 cells/mL on 15 g/mL poly-L-ornithine (Sigma). For the differentiation studies, cultures were maintained in DMF12 without growth factors (EGF and bFGF). Three days after the cells were plated, various concentrations of $A\beta_{42}$, in the different aggregation states, were added for 24 h. Four days later, immunocytochemical studies were performed wherein the number of cells expressing the marker for neurons (β-tubulin III) out of all the 4,6-diamino-2-phenylindole (DAPI)-positive cells was counted in random fields. For the proliferation studies, HPs were plated in DMF12 with growth factors (EGF and bFGF). One hour later, various concentrations of $A\beta_{42}$, in the different aggregation states, were added together with 1 μM of bromodeoxyuridine (BrdU) for 24 h. Proliferation of HPs was measured by BrdU incorporation. The effect of Wnt3a was evaluated by changing the DMF12 medium to Wnt3a or control L-cell-conditioned medium 1 h before adding $A\beta_{42}$. For glycogen synthase kinase 3 (GSK-3) inhibition, we added 100 μM of the GSK-3 selective inhibitor, L803-mts, developed in the laboratory of Prof. Hagit Eldar-Finkelman, Tel Aviv University (Plotkin *et al.* 2003) or glycerol 1 h before the addition of $A\beta_{42}$.

Immunofluorescence

Cells were fixed with 4% paraformaldehyde for 15 min in 4°C, permeabilized with a 0.15% Triton X-100 solution for 15 min, blocked with 10% goat serum for 1 h, and incubated with primary antibody overnight at 4°C. The following primary antibodies were applied: mouse anti-βIII tubulin (Tuj1) (1 : 500; Covance, Richmond, CA, USA), rat anti-glial fibrillary acid protein (1 : 500; Zymed, San Francisco, CA, USA), and mouse anti-glial fibrillary

acid protein (1 : 500; Sigma). Fluorescent-labeling Alexa Fluor 488 or 568 conjugated secondary antibodies against rabbit IgG and mouse IgG were used for detection (both 1 : 1000; Molecular probes, Eugene, OR, USA). Nuclear DNA was stained with DAPI (1 : 1000; Sigma). Cells were photographed using a fluorescence Olympus IX70-S8F2 microscope with a fluorescent light source (excitation wavelength, 330–385 nm; barrier filter, 420 nm) and a U-MNU filter cube (Olympus, Essex, UK).

Proliferation studies

Proliferation was measured using a BrdU cell proliferation kit (Chemicon, Temecula, CA, USA) with colorimetric detection, performed according to the manufacturer's instructions. Newly synthesized BrdU-DNA was identified by measuring absorbance at 450 and 570 nm with a BioTek PowerWave Microplate Reader (BioTek, Winooski, VT, USA).

RNA isolation and cDNA synthesis

Primary cultures were treated, and total RNA was isolated with a commercial TriReagent (Sigma). The amount and quality of RNA was determined with the ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA). First-strand cDNA synthesis was carried out with Super Script II RNase H-reverse transcriptase (Invitrogen) using a random primer.

Real-time PCR

The expression of related genes was quantified in the ABI Prism 7700 sequence detection system (Applied Biosystems, Carlsbad, CA, USA) using Platinum® SYBR® Green qPCR SuperMix UDG with ROX (Invitrogen), performed according to the manufacturer's instructions. PCR was performed in triplicate in optimized conditions: 50°C for 2 min, denaturation at 95°C for 2 min, followed by 40 cycles of 15 s at 95°C and 30 s at 60°C using the following primers: Mash1 (Fwd: TCTCCTGGGAATGGACTTTG; Rew: GGTTGGCTGTCTGGTTTGTT), NeuroD1 (Fwd: CCCGAGG-CTCCAGGGTTAT; Rew: CCCGCTCTCGCTGTATGATT), Ngn1 (Fwd: AGCTCACACTTTCTGCCAGG; Rew: CTTCGCCTT-GGTTTCCCT). No other products were amplified because melting curves showed only one peak in each primer pair. The expression of the gene of interest was quantitatively calculated against GAPDH using the $\Delta\Delta C_T$ method, as instructed in the user bulletin 2 of the ABI prism 7700 sequence detection system.

In-cell western

Cells were fixed with 4% paraformaldehyde for 20 min at 4°C, permeabilized with a 0.1% Triton X-100 solution for 15 min, blocked with 10% goat serum for 1 h, and incubated with primary antibody overnight at 4°C. The following primary antibodies were applied: mouse anti-active-β-catenin (1 : 400; Millipore, Billerica, MA, USA), rabbit anti-cleaved caspase 3 (1 : 500; Cell Signaling, Danvers, MA, USA), and mouse anti-β-actin (1 : 10 000; Sigma) or rabbit anti-emerin (1 : 4000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Secondary detection was carried out using two infrared-fluorescent-dye-conjugated goat antibodies: IRDye® 800CW and IRDye® 680LT (1 : 200; LI-COR Biosciences, Lincoln, NE, USA). The plates were imaged on an Odyssey infrared scanner (LI-COR) with a sensitivity of six in both the 700 and 800 nm wavelength channels. Data were acquired using Odyssey software (LI-COR).

Values of active-β-catenin and cleaved caspase 3 were background-subtracted from wells treated only with secondary antibody and then normalized to emerin or β-actin by dividing by the total DNA fluorescence signal.

Statistical analysis

Results were expressed as mean ± SEM. Immunopositive cells were counted for each antibody from three independent experiments done in triplicate. In each culture, ten 40× microscopic fields were counted. The number of positive cells was corrected for total cells and counted with DAPI staining. All analyses were performed using the SPSS, version 17 (SPSS, Chicago, IL, USA). Differences between two groups were assessed with Student's *t*-test. Differences between three or more groups were assessed by one-way analyses of variance (ANOVAS) followed by Scheffe's test. The level of significance was set at $p \leq 0.05$.

Results

Monomeric and oligomeric Aβ42 peptide affects HPs proliferation and differentiation

The effect of Aβ42 on neural progenitor cells differs by the source and developmental stage of the progenitor, the timing of treatment, and the Aβ aggregation state and concentration (Waldau and Shetty 2008; Lazarov and Marr 2010). Therefore, we used a well-controlled cultured model system of embryonic HPs. Transmission electron microscopy was used to identify the Aβ42 aggregation state (Fig. 1a).

Plating the progenitor cells in DM12 with growth factors supported their proliferation without allowing them to differentiate. Assessment of the proliferative effect of increased concentrations (0.1, 5, 10 μM) and aggregation states of Aβ42 showed that 24 h of HPs exposure to high concentrations (5 and 10 μM) of monomeric Aβ42 and a low concentration (1 μM) of oligomeric Aβ42 significantly increased the amount of incorporated BrdU compared with controls. The fibrillar state of Aβ42 did not affect proliferation (Fig. 1b).

Plating the progenitor cells in DMF12 without growth factors enabled them differentiate into both neurons and glia. Assessment of the effect of increased concentrations (0, 1, 5 and 10 μM) and aggregation states of Aβ42 for 24 h showed that a high concentration (10 μM) of monomeric Aβ42 and a low concentration (1 μM) of oligomeric Aβ42 decreased the number of Tuj1-expressing neurons compared to controls. In contrast, 5 μM of fibrillar Aβ42 caused a significant increase in the number of neurons (Fig. 1c).

Wnt3a increases the number of neurons in the progeny of Aβ42 treated HPs

The application of conditioned medium containing Wnt3a to differentiating HPs for 24 h (Fig. 2a) induced a threefold increase in the percentage of neurons compared with controls. When added with 1 μM of oligometric Aβ42, Wnt3a completely restored the neuron percentage in the Aβ42-treated
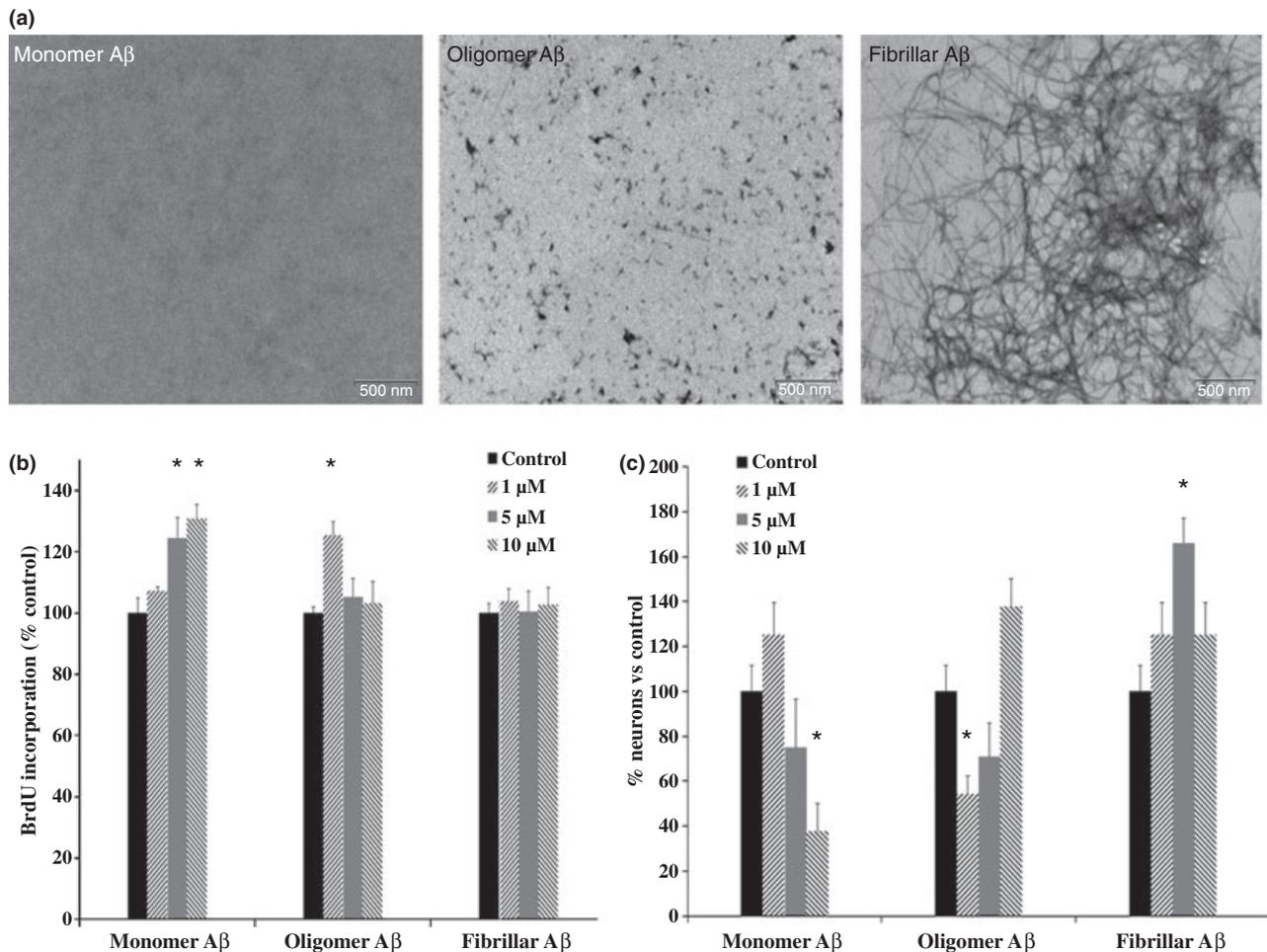
(a)

(b)

(c)

Fig. 1 Effect of three forms of Aβ$_{42}$ on the proliferation and differentiation of HPs. (a) Transmission electron microscopy images of three aggregation states of Aβ$_{42}$. (b) Cells were treated for 24 h with increased concentrations of Aβ$_{42}$ in the three aggregation states. BrdU incorporation was measured. High concentrations of monomeric Aβ$_{42}$ (5 and 10 μM) and a low concentration of oligomeric Aβ$_{42}$ (1 μM) increased HPs proliferation. (c) Cells were treated for 24 h with increased concentrations of Aβ$_{42}$ in the three aggregation states. Four days later, cells were immunolabeled with Tuj1. A high concentration of monomeric Aβ$_{42}$ (10 μM) and a low concentration of oligomeric Aβ$_{42}$ (5 μM) decreased neuronal differentiation. Results show mean ± SEM of three experiments done in triplicate. Statistical significance of *t*-test compared to control, *$p < 0.05$.

cells and even increased it threefold compared with controls. In parallel, Wnt3a induced a significant, 25%, reduction in astrocytes in the untreated and Aβ$_{42}$-treated HPs (Fig. 2b).

GSK–3 inhibition recapitulated Wnt3a–induced HPs differentiation

The addition of 100 μM of the GSK-3 selective inhibitor L803-mts to the culture led to a significant, twofold, enhancement of the neuron percentage in both the untreated and Aβ$_{42}$-treated HPs (Fig. 3).

Wnt signaling components are affected in HPs exposed to Aβ$_{42}$

Wnt, insulin, integrins, and growth factors signal through GSK-3β. However, in canonical Wnt signaling, GSK-3β phosphorylates β-catenin and targets it for degradation (Jope and Johnson 2004; Hur and Zhou 2010). Wnt binds to Frizzled receptors on the cell surface and blocks GSK-3β, thereby elevating intracellular active β-catenin levels. Accumulated β-catenin moves into the nucleus where it binds to and activates the transcription of Wnt target genes. We used In-cell Western analysis to examine possible changes in Wnt signaling components in HPs exposed to Aβ$_{42}$. The findings showed that Wnt3a significantly increased the level of active β-catenin protein in both untreated and Aβ$_{42}$-treated cells (Fig. 4a). Real-time PCR tests to determine changes in transcription of proneural genes revealed a down-regulation in mRNA levels of NeuroD1, Ngn1, and Mash1 genes. Gene expression was up-regulated after exposure of the untreated and Aβ$_{42}$-treated cells to Wnt3a (Fig. 4b).
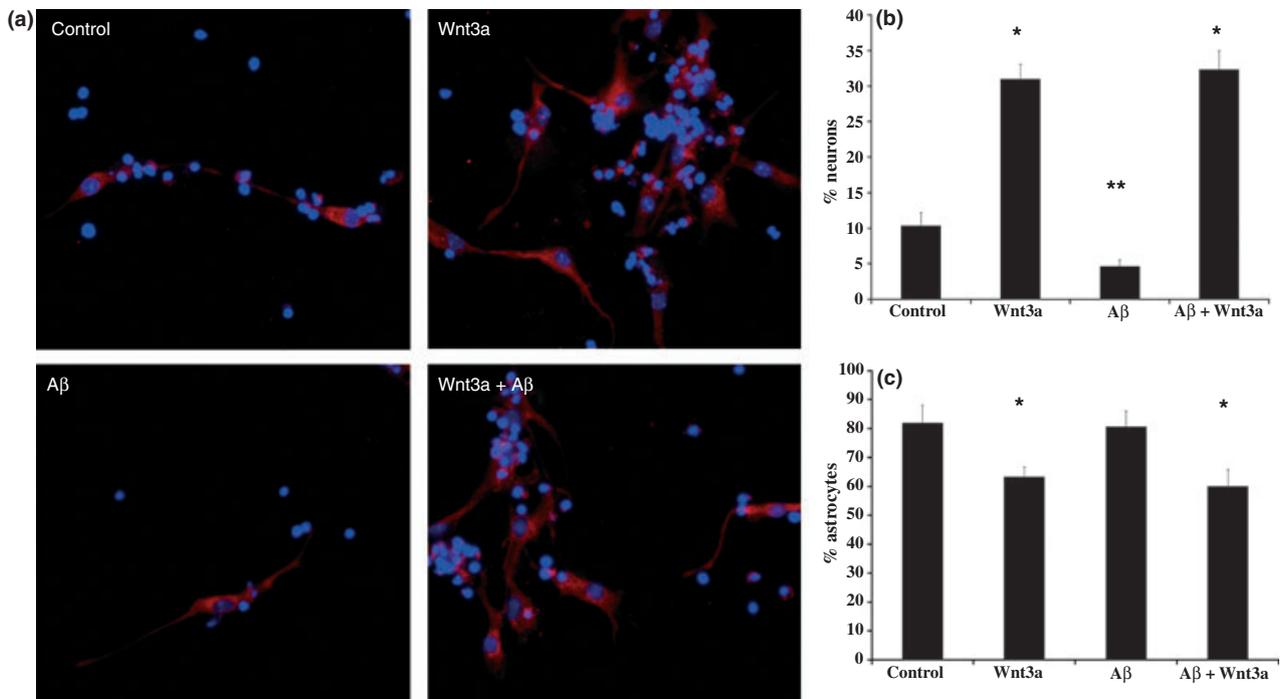
Fig. 2 Neurogenic effect of Wnt3a on HPs. (a) Representative micrograph of immunolabeled HPs after differentiation. HPs were induced to differentiate by withdrawing growth factors; 3 days later the medium was changed to L-cell-conditioned medium (control) or Wnt3a-conditioned medium, with or without $A\beta_{42}$ for 24 h, followed by immunolabeled with Tuj1. (b) Wnt3a increased the neuronal percent- age threefold compared with controls and enhanced $A\beta_{42}$-treated HPs differentiation threefold compared with controls. (c) Glial fibrillary acid protein labeling showed that Wnt3a reduced the astrocyte percentage in $A\beta_{42}$-treated and untreated differentiated HPs. Results show mean ± SEM of three experiments done in triplicate. Statistical significance on one-way ANOVA followed by Scheffe's test, *$p < 0.05$.
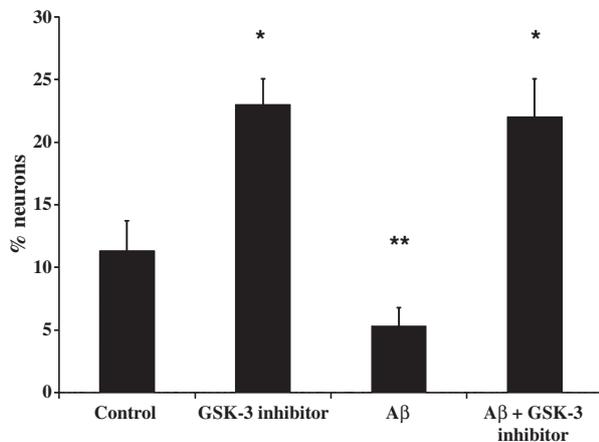

Fig. 3 Effect of GSK-3 inhibition The addition of the GSK-3 specific inhibitor (L803-mts) led to a twofold increase in neuron percentage in the $A\beta_{42}$-treated and untreated HPs compared with controls. Results show mean ± SEM of three experiments done in triplicate. Statistical significance on one-way ANOVA followed by Scheffe's test, *$p < 0.05$.

Wnt3a does not reduce apoptosis in $A\beta_{42}$ treated HPs progeny

The Wnt3a-induced increase in the percentage of neurons in the $A\beta_{42}$-treated HPs progeny raised the possibility that Wnt canonical activation affects cell fate. Therefore, to determine if the percentage of neurons increased because Wnt signaling eliminated $A\beta_{42}$-induced premature neuronal death, we measured levels of cleaved caspase 3. The findings showed the same level of cleaved caspase 3 in the $A\beta_{42}$-treated HPs and the controls. Moreover, there was no significant change when $A\beta_{42}$-treated cells were exposed to Wnt3a (Fig. 5).

Discussion

It has been established that the clinical severity of AD is poorly correlated with the amount of amyloid plaques that are composed of the fibrillar state of $A\beta_{42}$ (Arriagada *et al.* 1992; Bussière *et al.* 2002). Therefore, studies of the effect of $A\beta$ on neurogenesis must take the amyloid aggregation state into consideration. Accordingly, this study showed that exposure to monomeric and oligomeric $A\beta_{42}$ increases the proliferation of HPs.

Interestingly, monomeric and oligomeric $A\beta_{42}$ also caused decreased neurogenesis. Previous studies suggested a correlation between cell proliferation and survival (Ciaroni *et al.* 2002), with the survival rate increasing as the inhibition of proliferation decreases. However, the findings in our system cannot be explained by intrinsic regulation because the
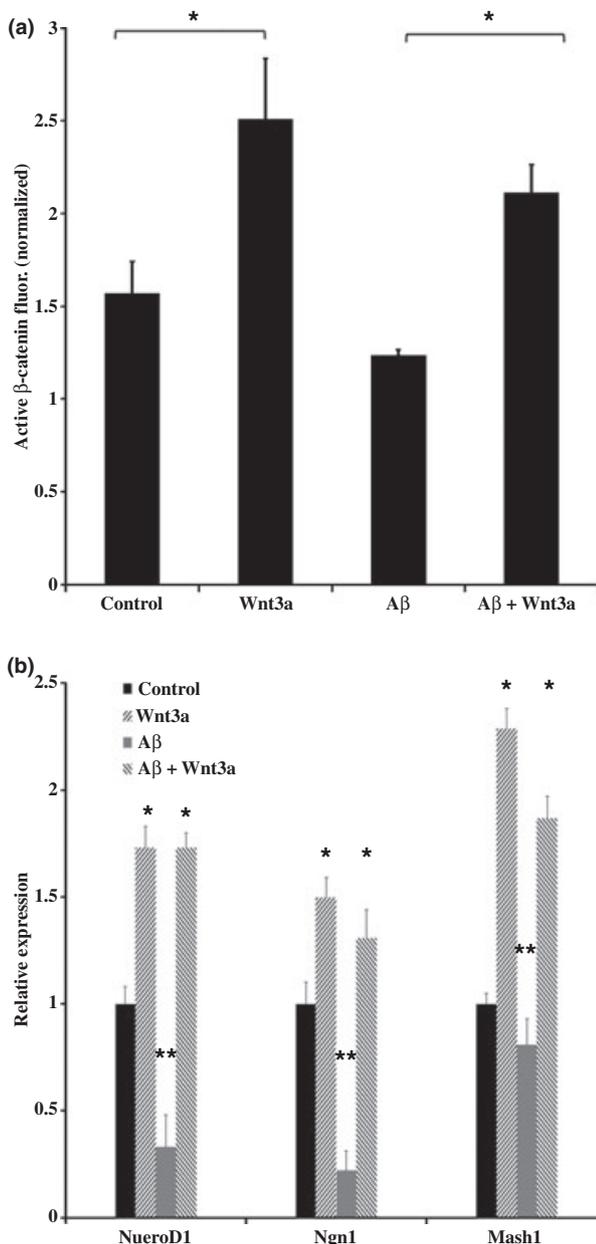
Fig. 5 Effect of Wnt3a on level of cleaved caspase 3 In-cell Western protocol yielded no change in the level of cleaved caspase 3 in Aβ$_{42}$-treated HPs. Wnt3a did not change the level of cleaved caspase 3 in Aβ$_{42}$-treated or untreated cells. Results show mean ± SEM of three experiments done in triplicate. Statistical significance on one-way ANOVA followed by Scheffe's test, *$p < 0.05$.

Fig. 4 Effect of Wnt3a on active β-catenin signaling and proneural gene transcription. (a) In-cell Western experiments showed that Wnt3a increased active β-catenin levels in differentiated HPs compared with control. A similar increase was found in Aβ$_{42}$-treated HPs. (b) The relative expression of NeuroD1, Ngn1 and Mash1 was quantified with real-time PCR in differentiated HPs. The expression was normalized to the respective GAPDH. Aβ$_{42}$ significantly reduced the relative expression of proneural genes and Wnt3a increased it in Aβ$_{42}$-treated and untreated HPs. Results show mean ± SEM of three experiments done in triplicate. Statistical significance in one-way ANOVA followed by Scheffe's test, *$p < 0.05$.

experiments were conducted separately, with each differentiation starting with the same number of cells. Furthermore, differentiation increased even when proliferation remained
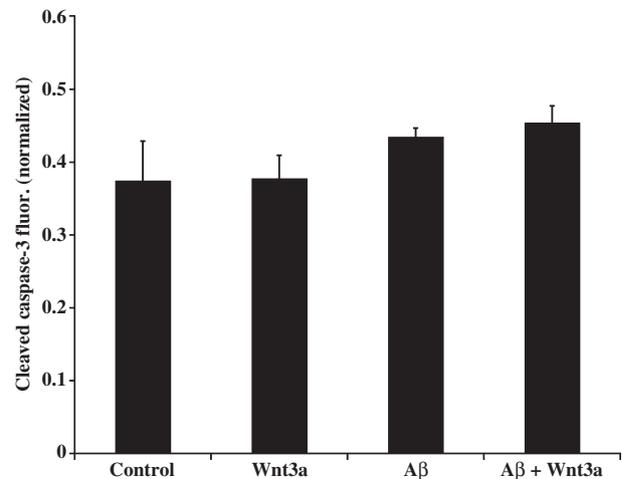
unchanged (in the presence of 5 μM fibrillar Aβ$_{42}$). Therefore, we presume the differentiation effect of Aβ$_{42}$ was exerted directly on the post-mitotic progenitor cells, and the changes observed were unrelated to a cell environmental effect.

In a study of the effect of Aβ$_{42}$ on neural stem cells isolated from the subventricular zone of adult mice, Heo *et al.* (2007) found that monomeric Aβ$_{42}$ inhibited cell proliferation and differentiation; oligomeric Aβ$_{42}$ enhanced proliferation and differentiation; and fibrillar Aβ$_{42}$ decreased proliferation with no effect on neurogenesis. The discrepancy from our results might be explained by a different effect of Aβ$_{42}$ on stem cells from different sources. Given that the hippocampus is one of the first and main areas affected in AD, when other areas remain unaffected (Caselli *et al.* 2006), our finding in hippocampal cells may promote a deeper understanding of the link between the pathogenesis of AD and neurogenesis. Our data correspond to another study on neural progenitor cells derived from P0 mice hippocampi in which fibrillar Aβ$_{42}$ caused an increase in neurogenesis, although oligomeric Aβ$_{42}$ did not (López-Toledano and Shelanski 2004).

Previous studies reported a connection between Aβ toxicity and loss of Wnt signaling, both *in vitro* and *in vivo*. The addition of Aβ$_{40}$ to neuronal cell cultures led to reduced levels of β-catenin, and the effect was reversed with Wnt3a conditioned medium (Kwak *et al.* 2006). Moreover, lithium treatment of rats injected with Aβ$_{40}$ fibrils rescued β-catenin levels, thereby preventing neurodegeneration and lessening the deficit in spatial learning (De Ferrari *et al.* 2003). In our study, Aβ$_{42}$ treatment reduced proneural gene expression of

the HP, but not β-catenin levels. Aβ has been shown to bind to the Frizzled cysteine-rich domain at or in close proximity to the Wnt-binding site and to inhibit the canonical Wnt signaling pathway (Magdesian *et al.* 2008). Therefore, the mechanism responsible for alterations in Wnt signaling may be directly related to Aβ.

Wnt signaling enhances adult neurogenesis *in vivo* and *in vitro* (Michaelidis and Lie 2008; Inestrosa and Arenas 2010) and is involved in regulating neurogenesis in AD. A recent study reported reduced neurogenic capabilities of glial progenitor cells derived from the cortex of patients with AD which correlated with elevated GSK-3β levels and increased phosphorylation of β-catenin. The induction of β-catenin over-expression in these cells increased neuronal differentiation. $A\beta_{42}$ treatment of glial progenitor cells from healthy patients led to reduced levels of proneural gene expression and a reduction in non-phosphorylated β-catenin (He and Shen 2009). Our study showed a similar effect in HP treated with oligomeric $A\beta_{42}$. The addition of Wnt3a completely restored the number of differentiated neurons and even increased it threefold compared with controls. This finding was repeated when a GSK-3 selective inhibitor was used.

In our study, oligomeric $A\beta_{42}$ had no effect on astrocyte differentiation, although others previously reported that soluble amyloid precursor protein decreases neurogenesis in AD by shifting the cell fate of neural stem cells to glial instead of neuronal lineage (Kwak *et al.* 2006). However, Wnt3a reduced the astrocyte number in the HPs, whether treated with $A\beta_{42}$ or not. Similarly, in a study of neurospheres from neonatal mouse forebrain, Wnt activation increased neuronal differentiation and blocked gliogenesis; inhibition of Wnt signaling increased gliogenesis at the expense of neurogenesis (Kunke *et al.* 2009). Thus, the Aβ-dependent reduction in neurogenesis may involve the loss of Wnt signaling, and Wnt3a activation of the canonical pathway may avert this process. The reduced neurogenesis in the absence of Wnt signaling could be caused by reduced cell survival, decreased proliferation of progenitors, or diminished neuronal cell fate decision. The lack of change in the level of cleaved caspase 3 in the $A\beta_{42}$-treated HPs, or in Wnt3a, indicates that Wnt signaling did not affect survival. Furthermore, the lack of difference in proliferation of the $A\beta_{42}$-treated HPs under the various differentiation conditions (data not shown) or after the addition of Wnt3a ruled out the possibility of decreased proliferation. Therefore, we suggest that Wnt3a directs neuronal fate to the neuronal lineage, and that changes in neurogenesis because of $A\beta_{42}$ or Wnt signaling also involve changes in gliosis.

Cumulative evidence suggests that reduced neurogenesis is implicated in cognitive deficits and that learning and memory processes depend on the generation of new neurons in the dentate gyrus (Deng *et al.* 2010). Studies reported that allopregnanolone reversed the neurogenic and cognitive deficits typical of male 3 × TgAD mice before the onset of overt AD pathology (Wang *et al.* 2010) and that inhibiting Wnt signaling in the dentate gyrus of adult male rats using dominant-negative Wnt caused decreased long-term retention of spatial memory in the water maze task and in a hippocampus-dependent object recognition task (Jessberger *et al.* 2009). Taken together with our study, these findings raise the possibility that delivery of drugs capable of modulating Wnt signaling to neural stem cells may serve as a novel strategy for overcoming neuronal loss and reduced the neurogenesis in AD. Additional studies, especially *in vivo*, are needed to evaluate the clinical implications of these findings.

Acknowledgements

References

Arriagada P., Growdon J., Hedley-Whyte E. and Hyman B. (1992) Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42**, 631–639.

Bruel-Jungerman E., Davis S. and Laroche S. (2007a) Brain plasticity mechanisms and memory: a party of four. *Neuroscientist* **13**, 492–505.

Bruel-Jungerman E., Rampon C. and Laroche S. (2007b) Adult hippocampal neurogenesis, synaptic plasticity and memory: facts and hypotheses. *Rev. Neurosci.* **18**, 93–114.

Bussière T., Friend P., Sadeghi N. *et al.* (2002) Stereologic assessment of the total cortical volume occupied by amyloid deposits and its relationship with cognitive status in aging and Alzheimer's disease. *Neuroscience* **112**, 75–91.

Caselli R., Beach T., Yaari R. and Reiman E. (2006) Alzheimer's disease a century later. *J. Clin. Psychiatry* **67**, 1784–1800.

Ciaroni S., Cecchini T., Ferri P., Ambrogini P., Cuppini R., Riccio M., Lombardelli G., Papa S. and Del Grande P. (2002) Impairment of neural precursor proliferation increases survival of cell progeny in the adult rat dentate gyrus. *Mech. Ageing Dev.* **123**, 1341–1352.

Dahlgren K., Manelli A., Stine W. J., Baker L., Krafft G. and LaDu M. (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. *J. Biol. Chem.* **277**, 32046–32053.

De Ferrari G., Chacón M., Barría M. *et al.* (2003) Activation of Wnt signaling rescues neurodegeneration and behavioral impairments induced by beta-amyloid fibrils. *Mol. Psychiatry* **8**, 195–208.

Deng W., Aimone J. and Gage F. (2010) New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat. Rev. Neurosci.* **11**, 339–350.

Drapeau E. and Nora Abrous D. (2008) Stem cell review series: role of neurogenesis in age-related memory disorders. *Aging Cell* **7**, 569–589.

Eckman C. and Eckman E. (2007) An update on the amyloid hypothesis. *Neurol. Clin.* **25**, 669–682, vi.

Haass C. and Selkoe D. (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat. Rev. Mol. Cell Biol.* **8**, 101–112.

He P. and Shen Y. (2009) Interruption of beta-catenin signaling reduces neurogenesis in Alzheimer's disease. *J. Neurosci.* **29**, 6545–6557.

Heo C., Chang K., Choi H. *et al.* (2007) Effects of the monomeric, oligomeric, and fibrillar Abeta42 peptides on the proliferation and differentiation of adult neural stem cells from subventricular zone. *J. Neurochem.* **102**, 493–500.

Hur E. and Zhou F. (2010) GSK3 signalling in neural development. *Nat. Rev. Neurosci.* **11**, 539–551.

Inestrosa N. and Arenas E. (2010) Emerging roles of Wnts in the adult nervous system. *Nat. Rev. Neurosci.* **11**, 77–86.

Jessberger S. and Gage F. (2009) Fate plasticity of adult hippocampal progenitors: biological relevance and therapeutic use. *Trends Pharmacol. Sci.* **30**, 61–65.

Jessberger S., Clark R., Broadbent N., Clemenson G. J., Consiglio A., Lie D., Squire L. and Gage F. (2009) Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. *Learn. Mem.* **16**, 147–154.

Jope R. and Johnson G. (2004) The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem. Sci.* **29**, 95–102.

Kunke D., Bryja V., Mygland L., Arenas E. and Krauss S. (2009) Inhibition of canonical Wnt signaling promotes gliogenesis in P0-NSCs. *Biochem. Biophys. Res. Commun.* **386**, 628–633.

Kwak Y., Brannen C., Qu T. *et al.* (2006) Amyloid precursor protein regulates differentiation of human neural stem cells. *Stem Cells Dev.* **15**, 381–389.

Lazarov O. and Marr R. (2010) Neurogenesis and Alzheimer's disease: at the crossroads. *Exp. Neurol.* **223**, 267–281.

Lie D., Colamarino S., Song H. *et al.* (2005) Wnt signalling regulates adult hippocampal neurogenesis. *Nature* **437**, 1370–1375.

López-Toledano M. and Shelanski M. (2004) Neurogenic effect of beta-amyloid peptide in the development of neural stem cells. *J. Neurosci.* **24**, 5439–5444.

Magdesian M., Carvalho M., Mendes F., Saraiva L., Juliano M., Juliano L., Garcia-Abreu J. and Ferreira S. (2008) Amyloid-beta binds to the extracellular cysteine-rich domain of Frizzled and inhibits Wnt/beta-catenin signaling. *J. Biol. Chem.* **283**, 9359–9368.

Michaelidis T. and Lie D. (2008) Wnt signaling and neural stem cells: caught in the Wnt web. *Cell Tissue Res.* **331**, 193–210.

Ming G. and Song H. (2005) Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223–250.

Mohs R., Schmeidler J. and Aryan M. (2000) Longitudinal studies of cognitive, functional and behavioural change in patients with Alzheimer's disease. *Stat. Med.* **19**, 1401–1409.

Nusse R., Fuerer C., Ching W., Harnish K., Logan C., Zeng A., ten Berge D. and Kalani Y. (2008) Wnt signaling and stem cell control. *Cold Spring Harb. Symp. Quant. Biol.* **73**, 59–66.

Plotkin B., Kaidanovich O., Talior I. and Eldar-Finkelman H. (2003) Insulin mimetic action of synthetic phosphorylated peptide inhibitors of glycogen synthase kinase-3. *J. Pharmacol. Exp. Ther.* **305**, 974–980.

Shruster A., Melamed E. and Offen D. (2010) Neurogenesis in the aged and neurodegenerative brain. *Apoptosis* **15**, 1415–1421.

Toledo E., Colombres M. and Inestrosa N. (2008) Wnt signaling in neuroprotection and stem cell differentiation. *Prog. Neurobiol.* **86**, 281–296.

Toni N., Laplagne D., Zhao C., Lombardi G., Ribak C., Gage F. and Schinder A. (2008) Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nat. Neurosci.* **11**, 901–907.

Waldau B. and Shetty A. (2008) Behavior of neural stem cells in the Alzheimer brain. *Cell. Mol. Life Sci.* **65**, 2372–2384.

Wang J., Singh C., Liu L., Irwin R., Chen S., Chung E., Thompson R. and Brinton R. (2010) Allopregnanolone reverses neurogenic and cognitive deficits in mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U S A* **107**, 6498–6503.

Zhao C., Deng W. and Gage F. (2008) Mechanisms and functional implications of adult neurogenesis. *Cell* **132**, 645–660.

Zhou C., Zhao C. and Pleasure S. (2004) Wnt signaling mutants have decreased dentate granule cell production and radial glial scaffolding abnormalities. *J. Neurosci.* **24**, 121–126.