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RESEARCH ARTICLE

Neurogenic effects of β-amyloid in the choroid plexus epithelial cells in Alzheimer's disease

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Abstract β-amyloid (Aβ) can promote neurogenesis, both in vitro and in vivo, by inducing neural progenitor cells to differentiate into neurons. The choroid plexus in Alzheimer's disease (AD) is burdened with amyloid deposits and hosts neuronal progenitor cells. However, neurogenesis in this brain tissue is not firmly established. To investigate this issue further, we examined the effect of Aβ on the neuronal differentiation of choroid plexus epithelial cells in several experimental models of AD. Here we show that Aβ regulates neurogenesis in vitro in cultured choroid plexus epithelial cells as well as in vivo in the choroid plexus of APP/Ps1 mice. Treatment with oligomeric Aβ increased proliferation and differentiation of neuronal progenitor cells in cultured choroid plexus epithelial cells, but decreased survival of newly born neurons. These Aβ-induced neurogenic effects were also observed in choroid plexus of APP/PS1 mice, and detected also in autopsy tissue from AD patients. Analysis of signaling pathways revealed that pre-treating the choroid plexus epithelial cells with specific inhibitors of TyrK or MAPK diminished Aβ-induced neuronal proliferation. Taken together, our results support a role of Aβ in proliferation and differentiation in the choroid plexus epithelial cells in Alzheimer's disease.

Keywords Choroid plexus · Amyloid · Neurogenesis · Transgenic mice · Alzheimer's disease patients

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Introduction

Neurogenesis is produced in a number of areas in the postnatal mammalian brain, primarily in the subventricular zone of the lateral ventricles, the olfactory bulb, and the granular cell layer of the hippocampus. Nevertheless, over the last decade it has been suggested that choroid plexus epithelial cells show proliferation and differentiation potential, hosting neural progenitor cells [1–3]. However, published results to date do not provide any definitive findings, and there is some skepticism over the potency of choroid plexus neurogenesis.

The choroid plexus is a specialized ependymal structure, its epithelial cells being of the same origin as ventricular subependymal cells. It is important to note that the subependymal or subventricular zone of the lateral ventricles is one of the three main brain areas where neurogenesis is still detectable in old age [4–6]. The discovery of a de novo production of neurons has introduced the possibility of a

new form of plasticity that could sustain memory processes. A growing body of evidence supports the view that promotion of adult neurogenesis improves pattern separation and spatial memory [7, 8]. In contrast, a decline in neurogenesis may underlie cognitive impairments associated with aging and disorders such as Alzheimer's disease (AD) [9, 10]. Although seemingly contradictory results have been reported in both murine and human studies [11], preserving or potentiating the production of new neurons has been regarded as a potential therapeutic strategy to delay or halt AD-linked cognitive decline. Furthermore, understanding the mechanisms of changes in neurogenesis observed at the initial and later stages of AD will contribute to the development of early AD biomarkers and reveal insights into the pathogenesis of AD.

The neuropathological characterization of AD involves a progressive deposition of β-amyloid protein (Aβ) protein in the brain parenchyma, often accompanied by Aβ deposition in cerebral blood vessels [12, 13] and choroid plexus [14, 15]. At the blood–cerebrospinal fluid barrier, choroid plexus plays a critical role in the support of neuronal function by clearing Aβ [15–19].

β-amyloid protein oligomers are thought to be an important source of neurotoxicity in AD [20–23], and this insult can influence the cognitive outcome [24]. Paradoxically, Aβ can also promote neurogenesis, both in vitro and in vivo, by inducing neural progenitor cells to differentiate into neurons [25, 26]. All of these findings suggest that Aβ can be modulating neural progenitor cell population in the choroid plexus.

To approach this question, we studied the effect of Aβ on the proliferation and differentiation of choroid plexus epithelial cells in vitro and in vivo. In the present study, we investigated the impact of Aβ-induced neurogenesis in the choroid plexus in AD. Thus, we studied the choroid plexus from rat primary cell cultures, double transgenic APP/Ps1 mice, and AD patients. We also characterized the possible signaling mechanisms by which Aβ exerts its effects on neurogenesis.

Materials and methods

Animals

We used the double-transgenic APP/PS1 mice, B6.Cg-Tg (APPSwe, PSEN1dE9)/J mouse line (Jackson Laboratory, Bar Harbor, ME, USA: stock no. 005864), which expresses human APP (Swedish mutation) and presenilin 1 with a deletion in exon 9 (APP/PS1). Both the APP/PS1 and the control non-transgenic littermates used were 12 months old. Animals were perfused transcardially either with saline buffer—for biochemical analysis—or 4 % paraformaldehyde in 0.1 M PB (pH 7.4)—for immunohistochemical analysis. Animal care and handling was in accordance with Spanish legislation (Spanish Royal Decree 1201/2005 BOE published October 21, 2005) and the guidelines of the European Commission for the accommodation and care of laboratory animals (revised in Appendix A of the Council of Europe Convention ETS123) and according to the Council Directive 2010/63/UE of September 22, 2010. The use of wild-type and transgenic animals was an absolute requirement for this project; however, experiments were designed to minimize the use of animals. All transgenic mice and non-transgenic littermates were group-housed in standard cages with fiber bedding, under a 12/12 h light/dark cycle and with ad libitum access to food and water.

BrdU administration and quantification of BrdU-positive cells

The 12-month-old double transgenic APP/PS1 mice and control mice were intraperitoneally injected with BrdU (50 μg/kg, Sigma) once a day for 7 days, and were killed 28 days later. To estimate the total number of BrdU-positive cells in the brain, we performed DAB staining for BrdU in adjacent sections in a one-six series of every animal, from bregma 1 mm, to the caudal end from bregma to 1 mm. The BrdU-positive cells in the choroid plexus were counted to estimate the total number of BrdU-positive cells in the entire choroid plexus as reported in previous studies [27, 28]. To determine the fate of dividing cells, all BrdU-positive cells across 4–6 sections per mouse were analyzed by confocal microscopy for co-expression with NeuN. The number of double-positive cells was expressed as a percentage of BrdU-positive cells.

Human samples

Choroid plexus samples from human autopsies were obtained from the Institute of Neuropathology Brain Bank IDIBELL-Hospital Universitari de Bellvitge (Hospitalet de Llobregat, Spain), and from Service of Pathology and Neuropathology, and Neurological Tissues Biobank of Vigo-Complejo Hospitalario Universitario de Vigo (Vigo, Spain), after the approval of the local ethics committee. Subjects were selected on the basis of post-mortem diagnosis of AD according to the neurofibrillary pathology and β-amyloid plaques [29]. Control cases were considered those with no neurological symptoms and with no lesions in the neuropathological examination. The time between death and processing was between 2 and 12 h. Choroid plexus samples from AD-related pathology ($n = 11$, stages V–VI), and age-matched controls ($n = 7$), were homogenized for immunoblot determination.

Immunoblotting

Proteins were isolated from brain tissue by standard methods, and Western-blot assay was performed as described

previously [16]. Densitometric analysis was performed using ImageJ software (NIH). Primary antibodies used included: goat anti-DCX (1:500, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), rabbit anti-calbindin (1:5,000 Millipore, Bedford, MA, USA), mouse anti-NeuN (1:5,000, Millipore), and mouse anti-β-actin (1:20,000, Sigma-Aldrich, St. Louis, MO, USA).

Immunohistochemistry

Mice brains were cut on a vibratome (Leica Microsystems, Wetzlar, Germany) at 40 μm, collected in cold 0.1 M PB, and incubated overnight with primary antibodies at 4 °C. All primary antibodies were diluted in PB 0.1 M containing 0.5 % BSA and 0.5 % Triton X-100. For BrdU labeling, brain sections were pre-treated with 2 N HCl at 37 °C for 30 min before incubation with primary antibody. Human brains were cut with a microtome, and 2-μm-thick sections were processed free-floating for immunohistochemistry. The following primary antibodies were used: mouse anti-BrdU (1:20,000, Hybridoma Bank), rat anti-BrdU (1:400, Chemicon), goat anti-DCX (1:250, Santa Cruz Biotechnology, Inc.), rabbit anti-calbindin (1:5,000 Millipore), and mouse anti-NeuN (1:500 Millipore). After overnight incubation, primary antibody staining was revealed using the avidin–biotin complex method (VECTASTAIN Elite ABC Kit, Vector Laboratories, Burlingame, CA, USA) and DAB chromogenic reaction (Vector Laboratories, Inc), or fluorescence-conjugated secondary antibodies: donkey anti-mouse IgG 488 (1:1,000; FluoProbes, Interchim), donkey anti-rat IgG 488 (1:1,000; FluoProbes, Interchim), chicken anti-goat IgG-Alexa 647 (Molecular Probes, Invitrogen), and DAPI nuclear staining (Sigma). Images were captured using a Zeiss LSM510 Meta scanning laser confocal microscope (Leica Microsystems).

Quantitative-PCR (qPCR)

RNA from the choroid plexus was extracted using the RNAspin mini kit (GE Healthcare, Waukesha, WI, USA). The DNA of the samples was obtained from 1 μg of RNA with a high capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA, USA) using the PCR program recommended by the manufacturer. DCX, calbindin, and GAPDH primers used were from Applied Biosystems. All samples were diluted 1:2 and run in triplicate. Standard curves for DCX, calbindin, and GAPDH with concentrations 1, 0.5, 0.25, and 0.125 μg were used to quantify DCX, calbindin mRNA. GAPDH was used as an internal control. Universal Taqman master mix from Applied Biosystems was used. Results were analyzed with the 7500 system SDS software (Applied Biosystems).

Cell culture and treatments

Choroid plexus cells from Wistar rats on postnatal day 3–5 (P3–P5) were prepared as described by Carro et al. [16]. Cells were grown 7 days before the treatment at 37 °C in a humidified atmosphere containing 5 % CO_2. Then, and after being serum starved for 2 h, the cells were incubated with fresh DMEM containing BrdU (50 μg/ml, Sigma), and the different reagents: $A\beta_{1-42}$ (5 μM; AnaSpec, Inc., San Jose, CA), genistatin (1 μM, Sigma), LY294002 (20 μM, Calbiochem), and PD098059 (20 μM, Calbiochem). After 24 h, the media were replaced with fresh media with or without $A\beta_{1-42}$ and the inhibitors, and incubated for 48 h, and 2 and 4 weeks.

Immunocytochemistry

Cell proliferation and differentiation were carried out in 24-well chamber slides. Cells were fixed with 4 % paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). For BrdU labeling, fixed cells were pre-treated with 2 N HCl at 37 °C for 30 min, blocked in 0.1 M PB containing 2 % bovine serum albumin (BSA) for 1 h at room temperature, and incubated with primary antibodies overnight at 4 °C. The following primary antibodies were used: mouse anti-BrdU (1:20,000, Hybridoma Bank), rat anti-BrdU (1:400, Chemicon), goat anti-doublecortin (DCX; 1:250, Santa Cruz Biotechnology, Inc.), mouse anti-NeuN (1:500, Millipore), mouse anti-GFAP (1:1,000; Sigma), rabbit anti-transthyretin (1:300, Santa Cruz Biotechnology, Inc.), and rabbit anti-calbindin (1:5,000 Millipore). After overnight incubation, primary antibody staining was revealed using fluorescence-conjugated secondary antibodies: donkey anti-mouse IgG 488 (1:1,000; FluoProbes, Interchim), donkey anti-rat IgG 488 (1:1,000; FluoProbes, Interchim), chicken anti-goat IgG-Alexa 647 (Molecular Probes, Invitrogen) and Texas Red goat anti-rabbit (1:1,000; Jackson ImmunoResearch, West Grove, PA, USA).

Cell proliferation was determined by performing immunocytochemistry for BrdU and counting the number of BrdU-labeled cells in each well under the 20× objective using a fluorescent Nikon microscope. Cell differentiation was determined by performing double labeling immunocytochemistry for BrdU and the neuronal markers DCX and NeuN, or the glial marker GFAP, and the procedure was performed as described for BrdU detection.

Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Differences between groups were analyzed by one-way analysis of variance. Post hoc comparisons between two groups were done with Student's *t* test. All calculations

were made using SPSS v15.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p < 0.05$.

Results

Cell proliferation and differentiation in choroid plexus in APP/PS1 mice

To determine whether amyloidogenic environment could modulate cell proliferation in the choroid plexus in vivo, APP/PS1 mice (12 months of age) were evaluated. This age was selected based on several previous studies that have demonstrated an increase in hippocampal cell proliferation and neurogenesis in the progressive stage of an AD phenotype [30, 31]. Cells incorporating BrdU were found in the choroid plexus of both control and APP/PS1 mouse groups (Fig. 1a). However, the number of $BrdU^+$ nuclei in the choroid plexus epithelial cells was significantly higher in APP/PS1 than in control mice ($p < 0.01$, Fig. 1b).

Cell proliferation in the choroid plexus of APP/PS1 mice was also confirmed by staining with DCX, the marker of immature neurons. Representative photomicrographs of DCX labeling are shown in Fig. 2a. The number of DCX^+ cells in the choroid plexus was significantly higher in APP/PS1 mice than in control mice ($p < 0.01$, Fig. 2b), supporting the hypothesis that an amyloidogenic environment stimulates proliferation of immature neurons in the choroid plexus. This finding was corroborated by measurement of DCX protein levels by Western blot. Immunoblotting analysis revealed a significant enhancement in DCX expression in choroid plexus from APP/PS1 mice compared to control mice ($p < 0.05$, Fig. 2c, d). Expression of mature neuronal markers was also tested. In contrast to DCX expression, 12-month-old APP/PS1 showed a significant reduction in calbindin ($p < 0.05$, Fig. 2c, e), and NeuN ($p < 0.01$, Fig. 2c, f) expression in the choroid plexus, compared to control mice. We also analyzed the genes encoding for these proteins. The present study confirmed the presence of mRNA for DCX and calbindin in the choroid plexus, as previously described [32], and we describe here for the first time their expression in APP/PS1 mice. Interestingly, the expression of genes encoding DCX and calbindin was unchanged in 12-month-old APP/Ps1 mice compared to age-matched control mice (Supplementary Fig. 1a, b).

To determine the extent of differentiation of survived BrdU-labeled cells, the brain sections from BrdU-treated APP/PS1 and control mice were processed for double-label immunohistochemistry with antibodies against BrdU and NeuN (Fig. 3a). Quantitative analysis showed that the number of BrdU-positive cells co-labeled with NeuN in the choroid plexus was significantly decreased in APP/PS1 mice compared to control mice ($p < 0.05$, Fig. 3b), representing an average of 3 % BrdU-positive cells colocalized with NeuN in control mice and less than 1 % in APP/PS1 mice. Taken together, our results support the hypothesis that an amyloidogenic environment initially might stimulate neurogenesis, but in due course survival of these newborn neurons would be reduced. In support of this last suggestion, apoptotic cell death was observed in choroid plexus from APP/PS1 mice [19].

Expression of neuronal markers in choroid plexus in AD patients

To verify the effect of amyloidogenic pathology on the expression of neuronal markers in choroid plexus from AD patients, this tissue was extracted from autopsies. With the help of Western blotting, we observed that, while DCX expression was unchanged in choroid plexus of AD patients

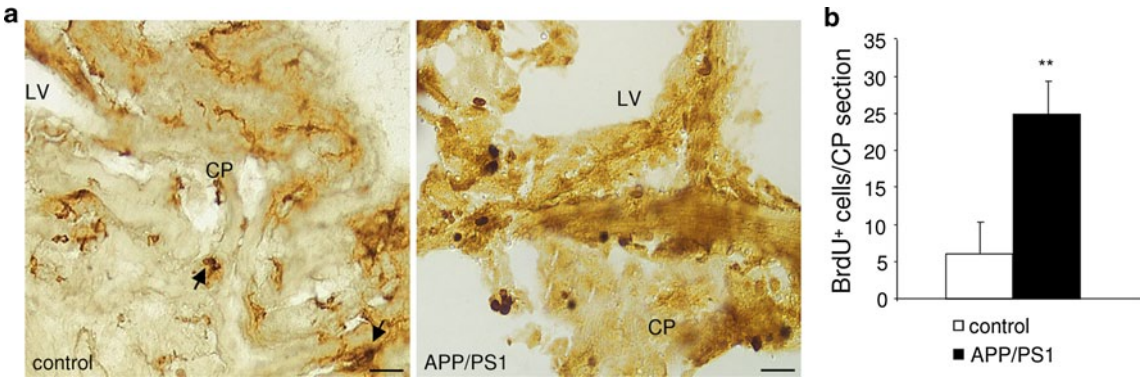

Fig. 1 Cell proliferation in the choroid plexus of APP/PS1 mice. **a** Representative $BrdU^+$ nuclei in the choroid plexus of control (*left*) and APP/PS1 (*right*) mice. **b** Quantitative analysis showing higher number of $BrdU^+$ nuclei in the choroid plexus of APP/PS1 mice compared with control non-transgenic mice. Data are expressed as mean ± SEM. $^{**}p < 0.01$, $n = 7$ mice per group. *LV* lateral ventricle; *CP* choroid plexus. *Scale bar* = 20 μm

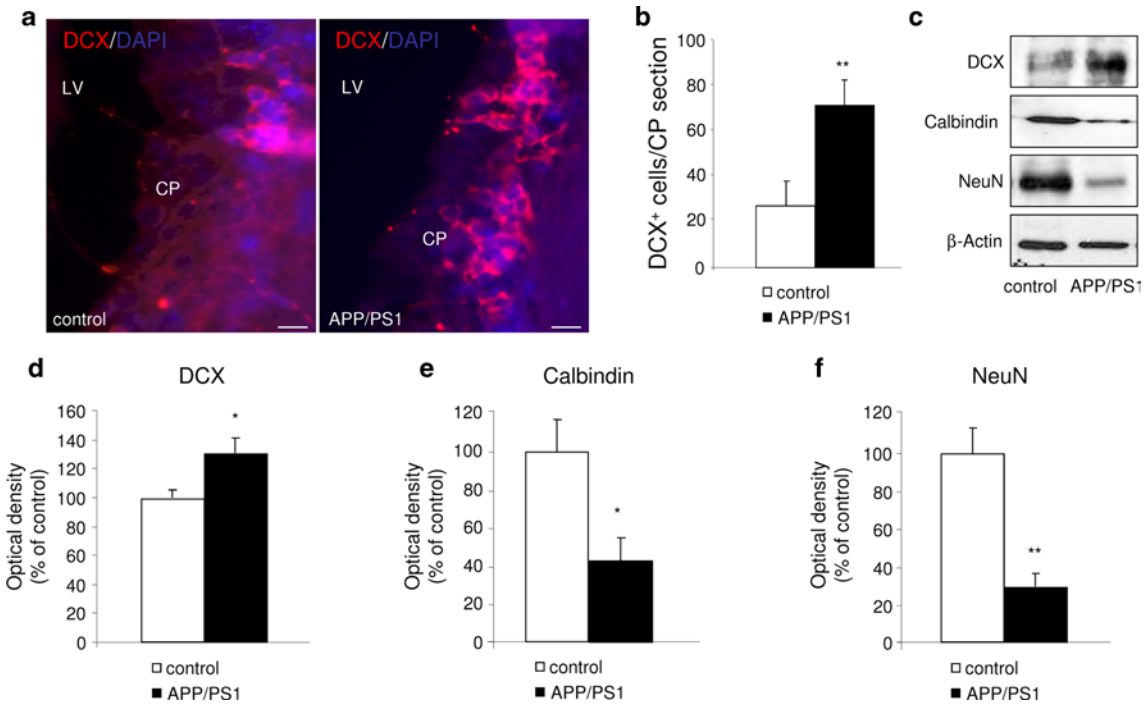

Fig. 2 Cell differentiation in the choroid plexus of APP/PS1 mice. **a** Photomicrographs show fluorescent DCX (*red*) staining of choroid plexus epithelial cells in control (*left*) and APP/PS1 (*right*) mice. Nuclei were counterstained with DAPI (*blue*). *Scale bars* = 20 μm. **b** The histogram revealed higher number of DCX^+ cells in the choroid plexus from APP/PS1 mice compared with control group. **c** Representative immunoblots showed the expression of DCX, calbindin, and NeuN in the choroid plexus homogenates of control and APP/PS1 mice. **d** Western-blot analysis showed that DCX expression was enhanced, whereas both calbindin (**e**) and NeuN expression (**f**) were reduced in the choroid plexus of APP/PS1 mice compared with control mice. Data are expressed as mean ± SEM. $*p < 0.05$, $**p < 0.01$, $n = 4$–7 mice per group. *LV* lateral ventricle; *CP* choroid plexus. *Scale bar* = 20 μm

compared to controls, a significant reduction in calbindin ($p < 0.05$), and NeuN expression ($p < 0.05$) was seen in the choroid plexus extracted from AD patients (Fig. 4a, b). These findings were confirmed by immunohistochemistry,

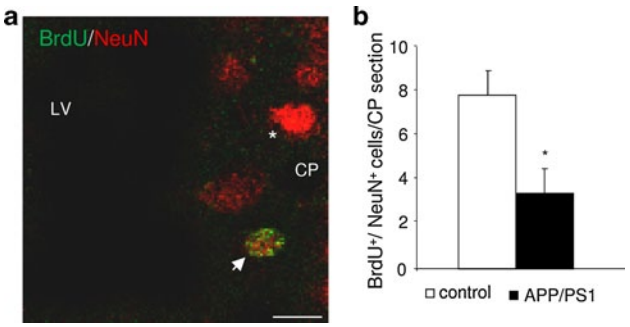

Fig. 3 Reduced neurogenic capacity in the choroid plexus of APP/PS1 mice. **a** Representative confocal microscopic image showing colocalization of BrdU (*green*) and NeuN (*red*) in the choroid plexus epithelial cells. *White arrow* indicates one double-labeled cell, and *white asterisk* indicates one $NeuN^+$ cell. **b** Quantitative analysis showed a reduction in the number of BrdU-labeled cells double labeled for NeuN in the choroid plexus from APP/PS1 mice. Data are expressed as mean ± SEM. $*p < 0.05$, $n = 5$ mice per group. *LV* lateral ventricle; *CP* choroid plexus. *Scale bar* = 20 μm

showing a dramatic decreased in the immunoreactivity of calbindin ($p < 0.001$, Fig. 4c, d), and NeuN ($p < 0.001$, Fig. 4e, f)-positive cells in AD choroid plexus.

Effects of $A\beta_{1–42}$ on cell proliferation and differentiation in choroid plexus cultures

Cultured choroid plexus epithelial cells were incubated with $A\beta_{1–42}$ for 48 h, and 2 and 4 weeks. Cellular proliferation, identified by BrdU reactivity, was vastly observed in choroid plexus epithelial cells cultured for 48 h and 2 weeks (Supplementary Fig. 2a, b). Analyses of the number of BrdU-labeled cells revealed an inverse time–response relationship between $A\beta_{1–42}$ and cell proliferation. Statistical analysis showed that the number of BrdU-labeled cells was significantly increased following $A\beta_{1–42}$ treatment for 48 h, whereas 2 and 4 weeks after $A\beta_{1–42}$ elicited no significant effects ($p < 0.05$, Fig. 5a, b).

Next, we investigated differentiation potential of cultured choroid plexus epithelial cells. Analysis of colocalization using confocal microscopy indicated that choroid plexus epithelial cell cultures primarily contained transthyretin-labeled cells (Fig. 6a) but also cells of neuronal lineage,

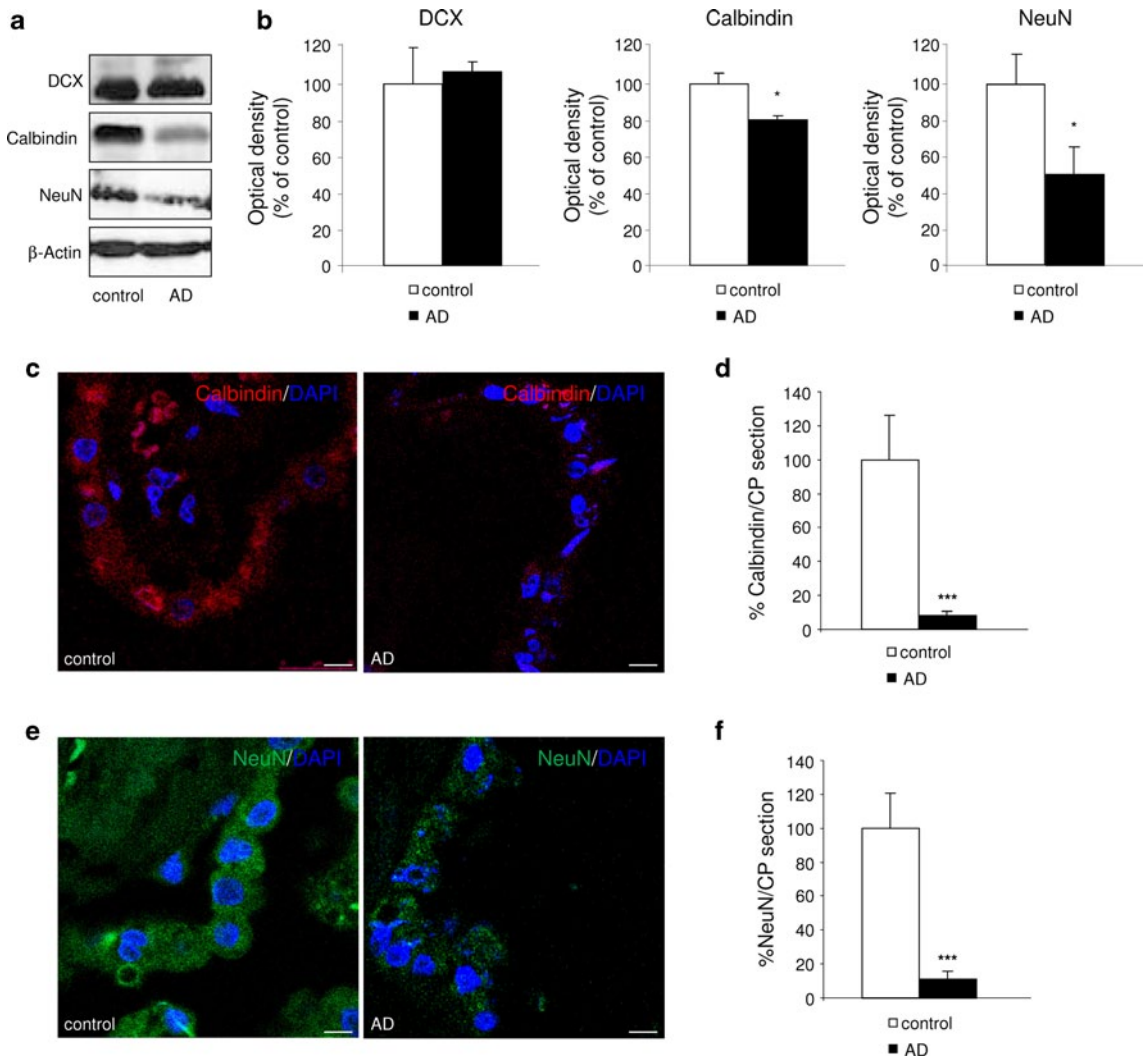

Fig. 4 Amyloidogenic effects on the neuronal marker expression in human choroid plexus. **a** Representative immunoblots showed the expression of DCX, calbindin, and NeuN in the choroid plexus homogenates from autopsy samples from control and AD patients. **b** Western-blot analysis showed that DCX expression was unchanged, whereas both calbindin and NeuN expression were reduced in the choroid plexus from AD autopsy subjects compared with control group. Data are expressed as mean ± SEM. $*p < 0.05$, $n = 4$–6 human autopsies per group. Representative confocal microscopic images showing **c** calbindin and **e** NeuN in human choroid plexus. Nuclei were counterstained with DAPI (*blue*). Quantitative analysis showed a dramatic reduction in the immunoreactivity of **d** calbindin, and **f** NeuN signals in choroid plexus from AD subjects. Data are expressed as mean ± SEM. $***p < 0.001$, $n = 4$ human autopsies per group. *Scale bar* = 10 μm

as demonstrated by the expression of phenotypic markers: DCX (Fig. 6b), NeuN (Fig. 6c), and GFAP (Fig. 6d).

To determine whether $A\beta_{1–42}$ affects cell differentiation in cultured choroid plexus epithelial cells, percentages of phenotypic markers were counted. Treatment with $A\beta_{1–42}$ resulted in significant changes in the percentages of BrdU-labeled cells that were positive for neuronal markers. Forty-eight-hour $A\beta_{1–42}$ treatment resulted in an increase number of DCX-positive cells that incorporated BrdU ($p < 0.05$, Fig. 6e). After long-term $A\beta_{1–42}$ treatments, NeuN-positive cells were evaluated. Double staining indicated that number of cells expressing BrdU and NeuN was significantly increased after 2 weeks of $A\beta_{1–42}$ treatment ($p < 0.01$, Fig. 6f), whereas this effect was inverted 4 weeks later ($p < 0.05$, Fig. 6g), suggesting a reduction in the survival of newly neuronal cells. This hypothesis is supported by a previous study where after exposure to $A\beta_{1–42}$, caspase-3 and caspase-9 expressions, and apoptotic cell death increased in choroid plexus cell cultures [19].

Neurogenic effects of $A\beta_{1–42}$ in choroid plexus cultures through TyrK/MAPK pathway

To evaluate the possible signal transduction pathways involved in Aβ-induced neurogenesis, we used several

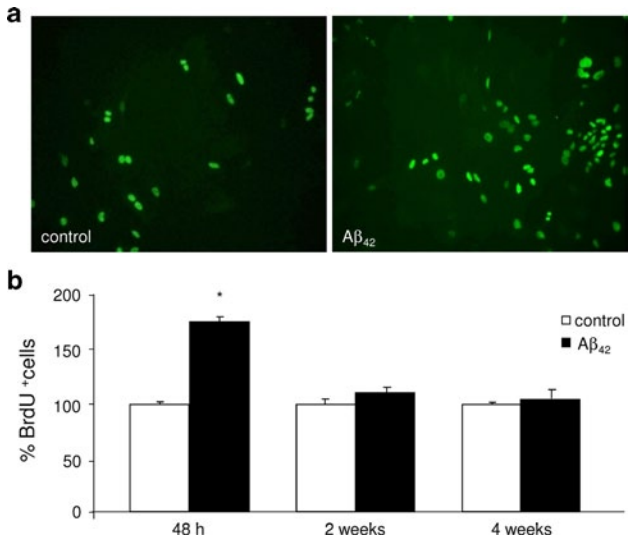

Fig. 5 $A\beta_{1–42}$ stimulates BrdU incorporation in cultured choroid plexus epithelial cells. **a** BrdU-labeled choroid plexus epithelial cells in control (*left*) and $A\beta_{1–42}$-treated cultured groups (*right*) at 48 h. **b** Quantitative analysis revealed an increase in BrdU-labeled cells after $A\beta_{1–42}$ treatment for 48 h. Data are expressed as mean ± SEM. $^*p < 0.05$. $n = 3$ independent experiments

inhibitors of pathways known to be involved in neurogenesis in other systems. These molecules included genistein, an inhibitor of tyrosine kinases (TyrK); PD098059, a selective mitogen-activated protein kinase (MAPK) inhibitor; and LY294002, a selective inhibitor of the phosphatidylinositol 3-kinase (PI3K).

Choroid plexus epithelial cells were simultaneously treated with oligomeric $A\beta_{1–42}$ and genistein for 2 weeks. At 1 μM concentration, genistein did not change the number of neurons observed versus controls. When $A\beta_{1–42}$ was added in the presence of genistein, the previously observed increase of neurons mediated by Aβ, (Fig. 6f), was abolished ($p < 0.01$, Fig. 7a). Similarly, treatment with the MAPK inhibitor PD098059 did not induce any change in total neurons in the culture but was able to inhibit the $A\beta_{1–42}$-induced increase in neurons ($p < 0.01$, Fig. 7b). In contrast, LY294002 was unable to inhibit $A\beta_{1–42}$-induced neurogenesis ($p < 0.01$, Fig. 7c).

Discussion

In the present study, our findings suggest that, both in cell cultures and in vivo, choroid plexus epithelial cells show cell proliferation and differentiation capability, and Aβ is able to modulate these neurogenic effects. We investigated neurogenesis in the choroid plexus in the context of amyloidosis using three experimental approaches: (1) choroid plexus cultures treated with oligomeric $A\beta_{1–42}$, (2) double transgenic APP/PS1 mice, (3) and AD patient autopsies. APP/PS1 mice generate several-fold more of the highly amyloidogenic $A\beta_{1–42}$ compared to $A\beta_{1–40}$ and thus develop

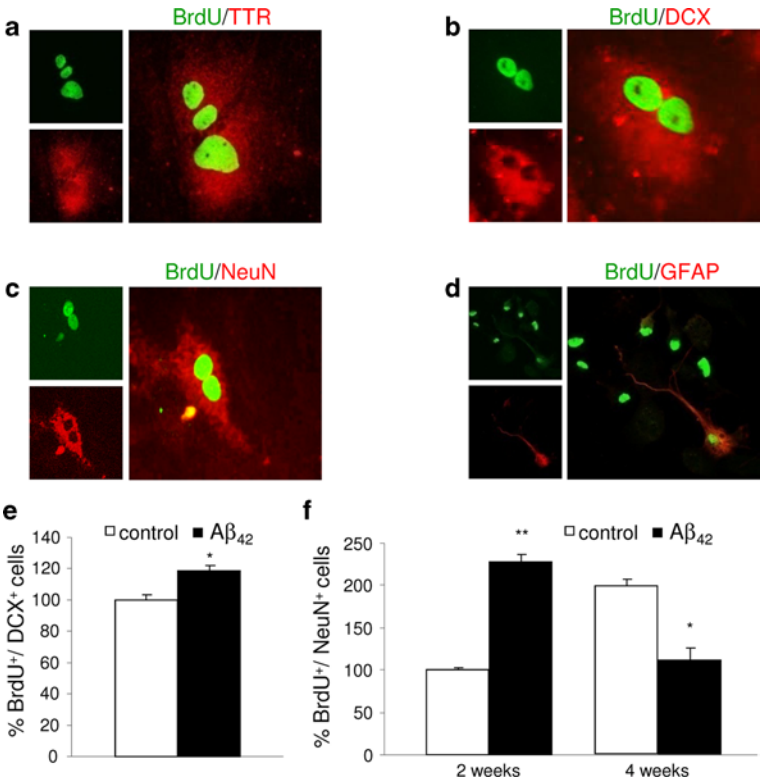

Fig. 6 Representative microscopic images showing colocalization of BrdU labeling (*green*) with immunoreactivity for phenotypic markers (*red*): **a** transthyretin (TTR), **b** doublecortin (DCX), **c** NeuN, and **d** GFAP. **e** Quantitative analysis indicating that the percentage of $BrdU^+$ and DCX^+ double-positive cells was increased by $A\beta_{1–42}$ treatment for 48 h. **f** Quantitative analysis showed an increased percentage of $BrdU^+$ and $NeuN^+$ double-positive cells in choroid plexus epithelial cells treated for 2 weeks with $A\beta_{1–42}$, whereas, this percentage was reduced after 4 weeks of treatment. Data are expressed as mean ± SEM. $^*p < 0.05$, $^{**}p < 0.01$. $n = 3$ independent experiments

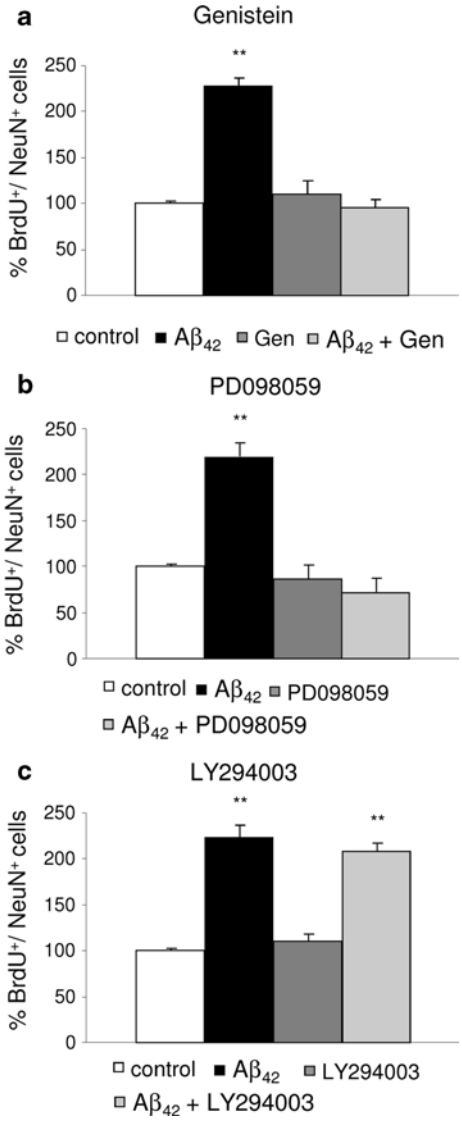

Fig. 7 Signaling pathways involved in the neurogenic effect of $A\beta_{1–42}$ in cultured choroid plexus epithelial cells. **a** A blockade of $A\beta_{1–42}$-induced neurogenic effects, considered as percentage of $BrdU^+$ and $NeuN^+$ double-positive cells, was observed with genistein, and with **b** PD098059, whereas no blockade was found with LY294002. Data are expressed as mean ± SEM. $^{**}p < 0.01$. $n = 3$ independent experiments

cerebral amyloidosis in hippocampus as early as 8 to 12 weeks [33].

Previous studies have described that choroid plexus epithelial cells had the capacity to proliferate and differentiate into other type of cells [1, 2, 34], based, at least in part, on the existence of a subpopulation of neural progenitor cells within the choroid plexus [3]. The proliferation of choroid plexus epithelial cells was reported in an experimental model of ischemic brain injury in the rat: BrdU-positive cells in the choroid plexus expressed GFAP and NeuN [2, 34]. The increase in the number of the BrdU-positive cells indicates that choroid plexus epithelial cells have the ability to proliferate when stimulated. It can be suggested that these cells have the ability to proliferate in response to certain injury-related stimuli. It is likely that the neural progenitor cells among choroid plexus epithelial cells are involved in proliferation following injury to the central nervous system.

It has been shown that several pathological conditions (ischemia, epilepsy, and trauma) seem to upregulate neural stem cell activity in the classical brain areas where neurogenesis is detectable in adult age, such as subventricular zone and dentate gyrus (for review, see Kuhn et al., [35]). These findings suggest that neural stem cell populations might be affected in AD. Thus, we investigated whether Aβ would modulate proliferation, survival, and differentiation of those neural progenitors cell within choroid plexus.

Numerous studies reported that the choroid plexus is considered an important source of trophic factors (for review, see Alvira-Botero and Carro, [15]), including several growth factors that are potent modulators of neurogenesis, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and vascular endothelial growth factor (VEGF), [27, 36–38], which may support neural progenitor cells within the choroid plexus by paracrine fashion. It is also known that in other brain areas, amyloid deposits stimulate the local accumulation of several growth factors, including BDNF and VEGF, which possess strong regulatory capacity for neurogenesis [39, 40]. The neurogenic effect of the Aβ peptide on hippocampal neuronal stem cells has also been reported [25]. In light of these findings, Aβ could be stimulating neural progenitor cells within the choroid plexus through prompting endogenous growth factor actions. This proliferation and differentiation stimulus in an amyloidogenic environment may also represent an endogenous neural replacement response to neurodegeneration and dysfunction. Indeed, in both AD patients and APP/PS1 mice, cell death in the choroid plexus has been reported [19]. As the APP/PS1 strain shows increased neurogenesis in the progressive stage of AD [30], it may reproduce the neurogenesis that is characteristic of AD patients [41].

In this study, we observed enhanced cell proliferation in the choroid plexus of APP/PS1 mice, compared to control mice. APP/PS1 mice overexpress APP but additionally express mutated PS1, and generate several-fold more of the highly amyloidogenic $A\beta_{1–42}$ compared to $A\beta_{1–40}$ and thus develop cerebral amyloidosis in hippocampus as early as 8 to 12 weeks. We detected an up-regulation of DCX-positive immature neurons and a reduction in mature neurons. This could be due to a dysfunction in the maturation processes or that the mature neurons undergo cell death. In vitro, we have observed that newly proliferative cells in the choroid plexus cultures differentiated into neurons, observed as an increased number of DCX-positive immature and NeuN-positive

mature neurons by 2 weeks after Aβ treatment. However, many of these newly born neurons die shortly after differentiation, and drastically diminished 4 weeks in vitro. This suggests that the neurons do mature but undergo cell death. This agrees with previous findings from our group where we found an increase in cell death in choroid plexus of APP/PS1 mice [19].

Furthermore, both our in vivo and in vitro findings are consistent with the study by Li et al. [42], in which intracerebroventricular infusion of $A\beta_{25-35}$ stimulated proliferation of progenitor cells in the hippocampal dentate gyrus of adult male mice, but a large population of the newborn cells proceeded to die in the second week after birth, a critical period for neurite growth. We suggest that these neurons might fail to obtain the sufficient quantity of neurotrophic factors, and would die by a process of programmed cell death. In agreement with this hypothesis, levels of neurotrophic factors are decreased in AD brains, including insulin-like growth factor I (IGF-I), BDNF, and VEGF [43–46]. We also reported Aβ-induced cell death in the choroid plexus from AD patients and APP/PS1 mice [19].

The work by López-Toledano and Shelanski [25] suggested a dependence of the neurogenic effect of Aβ on the state of aggregation of this peptide. According to this study, a possible interpretation of our data is that in earlier stages of AD, when the excess of Aβ is enough to form oligomeric but not fibrillar aggregates, the oligomers could activate a compensatory mechanism to replace lost or damaged neurons by increasing the differentiation of neuronal progenitors into new neurons [47, 48]. By later stages, Aβ deposits in choroid plexus are formed and become massive, and the balance could shift to fibrillar Aβ that could be more neurotoxic [19].

The impairment of neurogenesis observed with the aid of BrdU studies has been confirmed by quantitative Western-blot analysis of DCX, calbindin, and NeuN content, as immature and mature neuronal markers, respectively, in choroid plexus from APP/PS1 mice. This alternative approach for assessing neurogenesis in choroid plexus was also used in the subventricular-olfactory bulb system [49]. We also analyzed neurogenesis in choroid plexus from postmortem human brains by Western-blot and immunohistochemistry methods, as reported in the hippocampus of senile and presenile AD patients [41, 50]. Our results suggest that a microenvironment with chronic amyloid deposition can lead to a significant decrease in neurogenesis, consistent with data from our experiments with postmortem AD brains where a reduced expression of these markers in the choroid plexus has been observed.

One of the principal observations in this study was that neurogenic effects of Aβ in choroid plexus epithelial cells involved regulation of TyrK/MAPK signaling. The inhibition of neurogenesis by genistein suggests that tyrosine kinases are involved in this process. These kinases include the classical neurotrophin receptors that are essential in nervous system development (for review, see Chao et al. [51]). Recently, we have described the presence of p75NTR, a receptor for neurotrophins such as NGF and BDNF, in the choroid plexus [52]. We examined two signaling pathways downstream of TyrK. Inhibition of the PI3K system did not affect neurogenesis. In contrast, inhibition of the MAPK pathway blocked neurogenesis. The MAPK pathway has been implicated in the regulation of cell growth and proliferation, in differentiation, and in apoptosis [53, 54]. Numerous studies have described Aβ-induced activation of MAPK. In vivo injection of Aβ induces the activity of p38 MAPK in rat [55], and chronic exposure of human microglia to Aβ1-42 led to enhanced p38 MAPK expression [56]. All of these findings reinforce the implication of MAPK activation in the pathogenesis of AD, previously reported [57].

In summary, our present findings indicate that Aβ regulates the proliferation and differentiation of neural progenitor cells in the choroid plexus. However, our results suggest that amyloidogenic environment tends to down-regulate neurogenesis. Analysis of signaling pathways in vitro suggest that Aβ may stimulate the proliferation of newly born neurons through a mechanism that is dependent on MAPK activation. This study supports a novel role of choroid plexus in the processes of AD neuropathology, providing new insights into the mechanisms of neurogenic regulation.

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Conflict of interest The authors declare that they have no conflicts of interest.

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