Journal of Neuroscience Research

Journal of Neuroscience Research 90:559–567 (2012)

DNA Polymerase-β Mediates the Neurogenic Effect of β-Amyloid Protein in Cultured Subventricular Zone Neurospheres

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β-Amyloid protein (Aβ) is thought to be responsible for neuronal apoptosis in Alzheimer's disease (AD). Paradoxically, Aβ can also promote neurogenesis, both in vitro and in vivo, by inducing neural progenitor cells (NPCs) to differentiate into neurons. However, the mechanisms of Aβ-induced neurogenesis are unknown. Here we examined the role of DNA polymerase-β (DNA pol-β), a DNA repair enzyme that is required for proper neurogenesis during brain development and is also responsible for Aβ-induced neuronal apoptosis. In neurospheres obtained from the adult mouse subventricular zone (SVZ), the knockdown of DNA pol-β or its pharmacological blockade showed that the enzyme functioned both to repress proliferation of early nestin$^+$ progenitor cells and to promote the maturation of TuJ-1$^+$ neuronal cells. In neurospheres challenged with oligomers of synthetic $A\beta_{42}$, the expression levels of DNA pol-β were rapidly increased. DNA pol-β knockdown prevented the $A\beta_{42}$-promoted differentiation of nestin$^+$ progenitor cells into nestin$^+$/Dlx-2$^+$ neuroblasts. Moreover, when neurospheres were seeded to allow full differentiation of their elements, blockade of DNA pol-β prevented $A\beta_{42}$-induced differentiation of progenitors into MAP-2$^+$ neurons. Thus, our data demonstrate that $A\beta_{42}$ arrests the proliferation of a subpopulation of nestin$^+$ cells via the induction of DNA pol-β, thereby allowing for their differentiation toward the neuronal lineage. Our findings reveal a novel role of DNA pol-β in $A\beta_{42}$-induced neurogenesis and identify DNA pol-β as a key mechanistic link between the neurogenic effect of $A\beta_{42}$ on NPCs and the proapoptotic effect of $A\beta_{42}$ on mature neurons.

Key words: DNA polymerase-β; β-amyloid; neurogenesis; neurosphere; neural stem cell; Alzheimer's disease

Evidence for increased neurogenesis exists in the hippocampus and subventricular zone (SVZ) of Alzheimer's disease (AD) patients (Ziabreva et al., 2006). Enhanced neurogenesis has also been described in β-amyloid protein precursor (APP) transgenic mice before the appearance of other AD-related pathological features (López-Toledano and Shelanski, 2007), suggesting that it might be an important component of AD pathophysiology. Studies in vitro and in vivo have demonstrated that β-amyloid (Aβ), which is thought to be responsible for neuronal loss in AD, is able to drive the differentiation of neural progenitor cells (NPCs) toward neurons (López-Toledano and Shelanski, 2004; Calafiore et al., 2006). Aβ may induce neurogenesis early in the pathogenesis of AD, thereby resulting in exhaustion of the neural stem cell pool later in the life of AD patients (López-Toledano et al., 2010).

The mechanisms underlying the neurogenic effect of Aβ are unknown. Understanding the mechanisms of Aβ-induced neurogenesis might be relevant for exploiting the regenerative potential of the AD brain. Mature neurons respond to Aβ by initiating cell cycle re-entry, leading to Aβ-induced neuronal apoptosis (Morishima et al., 2001; Herrup et al., 2004). Specifically, it is the ectopic S phase that triggers the death of mature neurons (Copani et al., 1999). Neurons treated with Aβ undergo an unusual type of de novo DNA replication, mediated largely by the repair enzyme DNA polymerase-β (DNA pol-β), because they lack the major replicative enzyme, DNA pol-α (Copani et al., 2002). Knockdown of DNA pol-β prevents Aβ-induced DNA replication and neuronal death (Copani et al., 2002, 2006).

Contract grant sponsor: National Institutes of Health; Contract grant numbers: R01NS061983 and R01ES015988 (to W.D.); Contract grant sponsors: National Multiple Sclerosis Society (to W.D.), Shriners Hospitals for Children (to W.D.) and Italian Ministry of University and Research; Contract grant number: 105/04 (to A.C.).

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Received 10 May 2011; Revised 19 July 2011; Accepted 29 July 2011

Published online 4 November 2011 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jnr.22780

In NPCs, which express a canonical repertoire of replicative enzymes, DNA pol-β might instead function as a DNA repair enzyme involved in nonhomologous end-joining (Reichenberger and Pfeiffer, 1998) and gene recombination. In DNA pol-β-deficient mice, neuronal apoptosis occurs within the developmental period of neurogenesis (Sugo et al., 2000). Interestingly, when apoptosis is prevented by the concomitant deficiency of the proapoptotic factor p53, mice still display serious brain abnormalities (Sugo et al., 2004), suggesting that DNA pol-β is required for neuronal differentiation.

In the present study, by using NPCs derived from the adult mouse SVZ, we examine the hypothesis that the induction of DNA pol-β plays an important role in the neurogenic effect of Aβ. Our results reveals a novel role of DNA pol-β in Aβ-induced neurogenesis and identify DNA pol-β as a mechanistic link between the neurogenic effect of $A\beta_{42}$ on NPCs and the proapoptotic effect of $A\beta_{42}$ on mature neurons.

MATERIALS AND METHODS

Aβ Peptide Preparation

$A\beta_{42}$ was purchased from Bachem Distribution Service. $A\beta_{42}$ was prepared as previously described (Giuffrida et al., 2009). Briefly, $A\beta_{42}$ peptide was resuspended in dimethyl sulfoxide (DMSO) to 5 mM and then diluted to 100 μM in ice-cold Dulbecco's modified Eagle's medium-F12 (DMEM-F12; Gibco, Grand Island, NY). The suspension was allowed to oligomerize overnight at 4°C and was used at the final concentration of 1 μM in the presence of the ionotropic glutamate receptor antagonists MK-801 (1 μM) and DNQX (30 μM) to avoid the participation of the endogenous glutamatergic mechanism in subsequent experiments (Copani et al., 1999). Control experiments were conducted under the same conditions except for the addition of the peptide.

Neurosphere Preparation and Treatments

Neurosphere cultures were obtained from CD1 adult male mice. Brains were removed and placed in PIPES buffer (Fisher, Pittsburgh, PA). After dissecting the lateral walls of the lateral ventricle, cell suspensions were grown in DMEM-F12 supplemented with 10 mg/ml albumin, 5.2 μg/ml insulin, 0.63 ng/ml progesterone, 100 μg/ml transferin, 16.11 μg/ml putrescine, 2 mM glutamine, 0.5% glucose, 1% bovine serum albumin (BSA; all from Sigma, St. Louis, MO), 20 ng/ml epidermal growth factor (EGF), and 5 ng/ml of basic fibroblast growth factor (bFGF; both from Invitrogen, Carlsbad, CA) in 25-cm^2 flasks. Neurospheres that had been grown for 12–15 DIV were passaged and transferred into 35-mm dishes. When necessary for differentiation studies, neurospheres were plated onto dishes pretreated with poly-ornithine (20 μg/ml) and laminin (10 μg/ml), without EGF. The base analog 2′,3′-dideoxycytidine (ddC; 100 μM) was used as a selective inhibitor of DNA pol-β (Copani et al., 1999, 2002, 2006).

Use of Oligonucleotide Antisenses and Short Hairpin RNAs of DNA pol-β

The following "end-capped" phosphothioate oligonucleotide antisenses (obtained from MWG-Biotech) were used: pol β-As, 5′-tacttgtggatcgcctggct-3′; pol β-Sn, 5′-agccaggc gatccacaagta-3′ (bases 95–114 of the rat mRNA sequence NM017141). Cultures were treated with oligonucleotides (1.5 μM) 16 hr before the addition of Aβ. DNA pol-β short hairpin RNAs (shRNAs) cloned into pLKO.1 were purchased from Open Biosystem. The DNA pol-β shRNAs (TRCN0000077250 and TRCN0000077248), which that showed the highest levels of DNA pol-β knockdown among five plasmids that we tested, were used in this study. To establish stable expression, we infected the cells with a lentivirus-based shRNA expresser, and cells were selected with puromycin. An shRNA encoded for a scrambled sequence was used as a negative control.

Fluorescence-Activated Cell Sorting Analysis and Immunofluorescence Staining

Neurospheres were dissociated by incubation with 0.25% trypsin and collected by low-speed centrifugation after the addition of 20% fetal bovine serum (FBS). Cellular pellets were fixed in 70% ethanol and treated with RNAse (100 μg/ml) for 1 hr before propidium iodide staining (50 μg/ml for 10 min). Samples were simultaneously analyzed for cell cycle and apoptosis. DNA content and ploidy were assessed by using a CyAn flow cytometer, and cell-cycle distribution profiles were analyzed with the Multicycle AV software program. Apoptotic NPCs were scored from the area of hypoploid DNA preceding the G0/G1 DNA peak.

For fluorescence-activated cell sorting (FACS) analysis, suspended cells, prepared as described above, were fixed in 4% paraformaldehyde, permeabilized with 0.2% Triton X-100, and then stained overnight at 4°C with the following antibodies: mouse antinestin monoclonal antibody (Chemicon, Temecula, CA; 1 μg/ml), mouse anti-GFAP polyclonal antibody (Lab Vision; 1:500), mouse anti-PSA-NCAM (DHSB; 1:500), or rabbit anti-Dlx-2 polyclonal antibody (Chemicon; 1:400). The following secondary antibodies were used: Alexa Fluor 633 anti-mouse IgG (Invitrogen; 1:1,000) for the detection of nestin, GFAP, and PSA-NCAM and Alexa Fluor 488 anti-rabbit IgG (Invitrogen; 1:1,000) for the detection of Dlx-2.

Immunoblotting

Western blot analysis was carried out on total neurosphere protein extracts. Neurospheres were washed in phosphate-buffered saline (PBS) and resuspended in RIPA lysis buffer (Boston BioProducts) with a protease inhibitor cocktail (Roche) added. The cell suspension was sonicated, and protein concentration was determined using bicinchoninic acid assay (Sigma-Aldrich). Electrophoresis was performed in SDS-polyacrylamide gel, using 40 μg proteins per lane. The following primary antibodies were used: mouse monoclonal antinestin (Santa Cruz Biotechnology, Santa Cruz, CA; 1:400), rabbit polyclonal anti-GFAP (Chemicon; 1:500), rabbit polyclonal anti-TuJ-1 (Covance, Berkeley, CA; 1:1,000), rabbit polyclonal anti-MAP-2 (Chemicon; 1:300), mouse monoclonal anti-DNA pol-β (Lab Vision; 1:200), rabbit polyclonal anti-Dlx-2 (Chemicon; 1:500), mouse monoclonal anti-Bax (Santa Cruz Biotechnology; 1:200), rabbit polyclonal anti-p21 (Santa Cruz

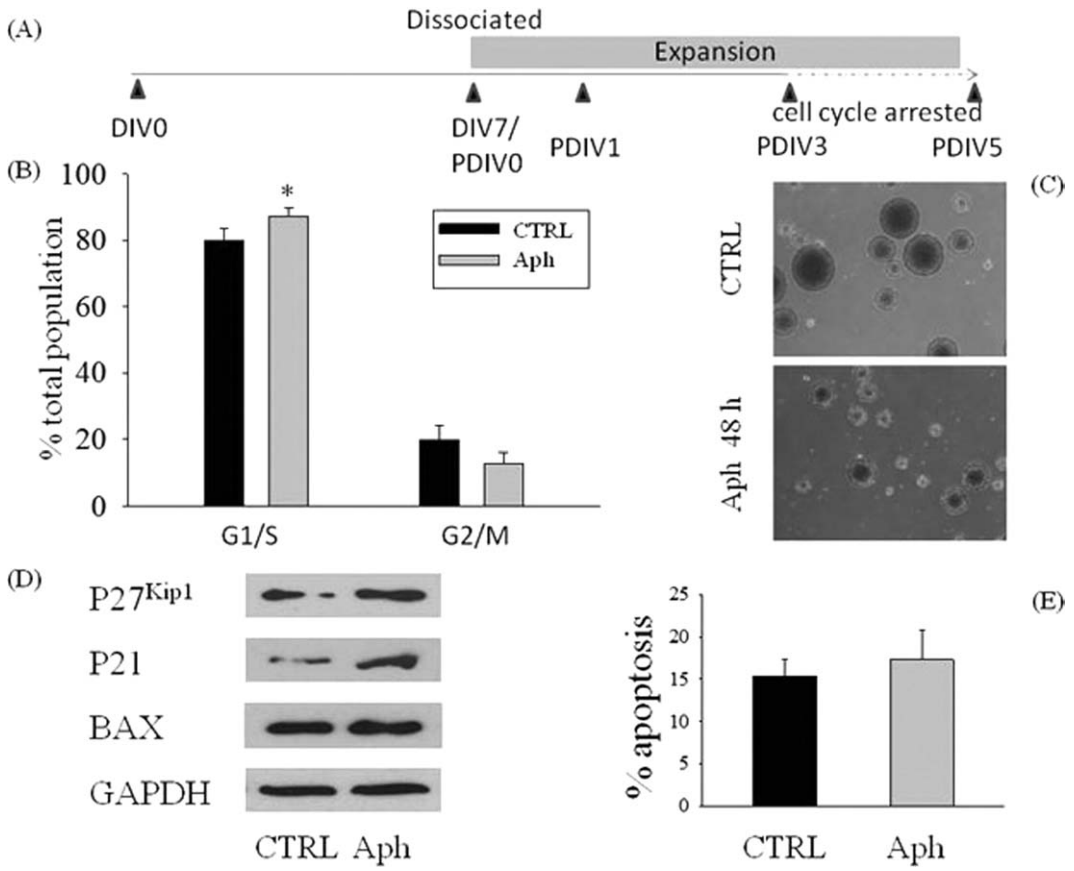


Fig. 1. Effect of cell cycle arrest of neurospheres induced by aphidicolin in the presence or absence of $A\beta_{42}$ on neural progenitor cells (NPCs). Schematic drawing of the experimental protocol (**A**). Neurospheres were grown as described in Materials and Methods. After 7 days in vitro (DIV 7), neurospheres were passaged, and NPCs were allowed to form secondary neurospheres. Three days after the first passage (PDIV 3), neurospheres were treated with 5 μM of the cell cycle inhibitor aphidicolin (Aph) for 48 hr. **C:** Phase-contrast microscopy of NPCs grown in vitro with or without Aph. Cell cycle distribution (**B**) and apoptosis (**E**) of neurospheres analyzed by flow cytometry 48 hr after treatment with Aph (5 μM). Values are mean ± SEM of five different determinations. $*P < 0.05$ (ANOVA followed by Tukey's post hoc test). CTRL, control. **D:** Representative blots of total protein extracts showing the increased expression of the cell cycle inhibitor proteins p21 and p27 after treatment with Aph and no difference in the proapoptotic protein Bax.

Biotechnology; 1:200), and mouse monoclonal anti-GAPDH (Santa Cruz Biotechnology; 1:1,000).

RESULTS

Aβ-Induced Neurogenesis and the Effect of Cell Cycle Arrest on Neurosphere Cultures From the SVZ

We previously reported that $A\beta_{42}$ induced neural progenitors of the adult mouse SVZ to acquire a neuronal phenotype (Calafiore et al., 2006), using the highly polysialated form of NCAM (PSA-NCAM) as a marker of neuronal-lineage cells. We noted that, in suspended neurospheres derived from the SVZ, $A\beta_{42}$-induced neurogenesis was coupled with an increased expression of the cyclin-dependent kinase (CDK) inhibitors p21 and $p27^{kip1}$, which promoted cell cycle arrest in the G1 phase (Calafiore et al., 2006). This observation raised a fundamental question about whether Aβ-induced neurogenesis is due to cell cycle arrest of the SVZ NPCs or direct induction of NPC differentiation into the neuronal lineage. Thus, we first examined whether cell cycle arrest per se, in the absence of $A\beta_{42}$, would be sufficient to promote the differentiation of NPCs into the neuronal lineage. We compared cells treated with and without a commonly used cell cycle blocker, aphidicolin (Aph; Sheaff et al., 1991; Jackman and O'Connor, 2001). Secondary neurospheres were exposed to 5 μM Aph at 3 days after expansion and then were allowed to grow for 48 hr (Fig. 1A). Under this condition, FACS analysis indicated that Aph modified the cell cycle distribution by arresting about 13% of the cells in the G1/S phases (Fig. 1B). Phase-contrast microscopic image of cultures at day 5 showed a dramatic reduction of the neurosphere size in cultures treated with Aph compared with controls (Fig. 1C). To quantify the effects of Aph treatment on death of NPCs, we assessed the levels of apoptosis by cytofluorimetric analysis (Darzynkiewicz et al., 1992;

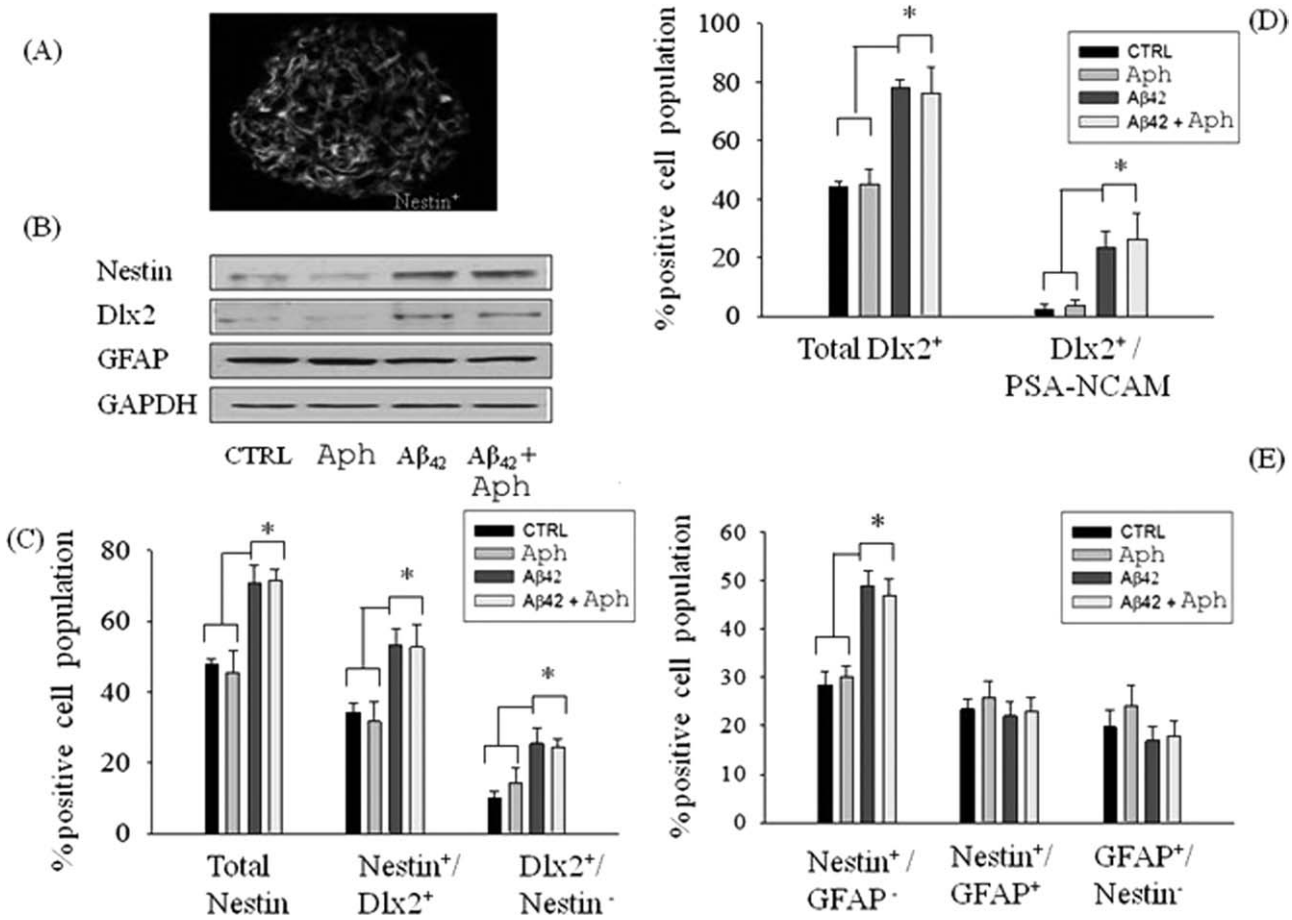


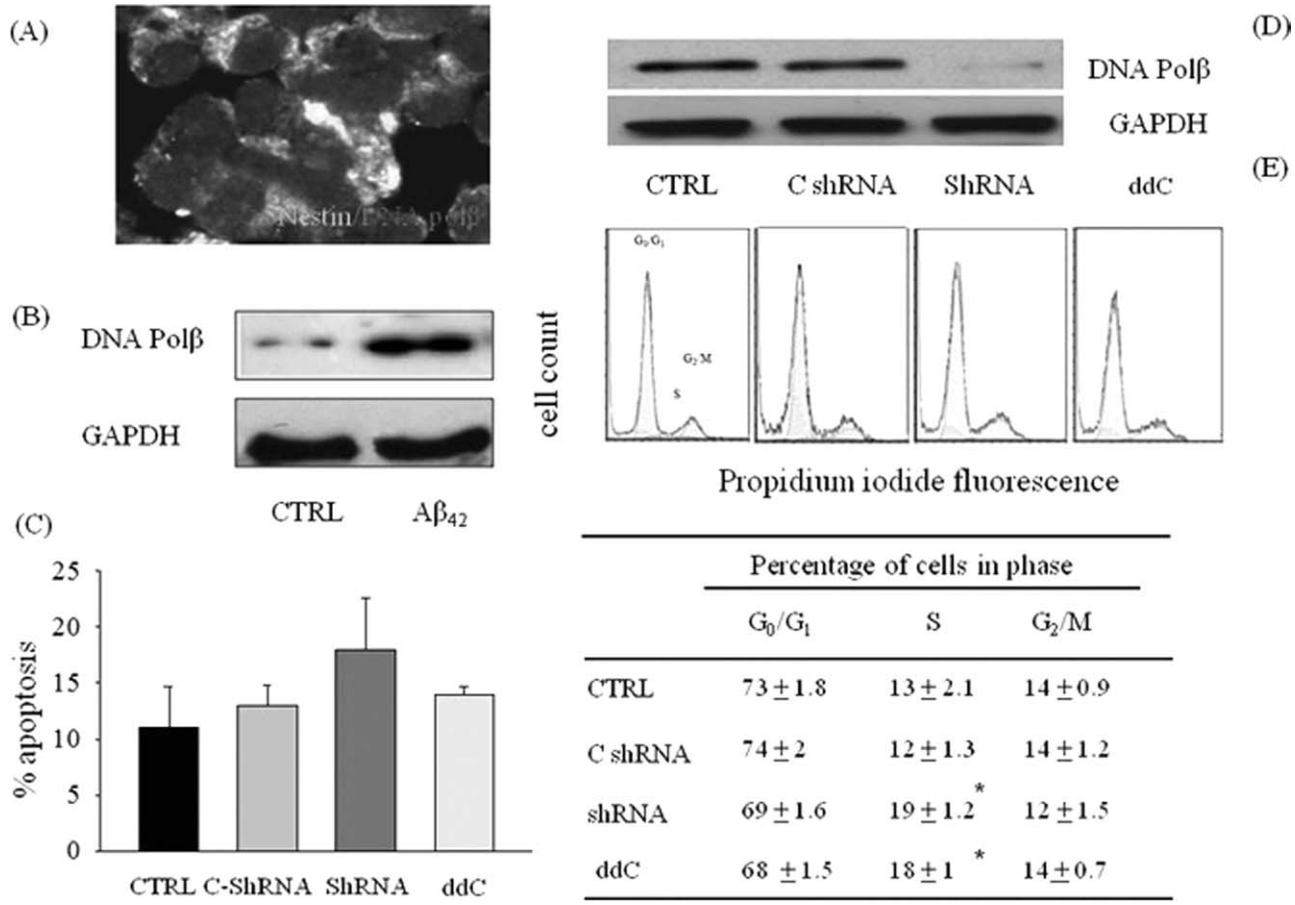
Fig. 2. NPC differentiation in the presence or absence of $A\beta_{42}$ was not affected by pharmacological blockade of the cell cycle. Confocal image of neurospheres showing immunoreactive nestin$^+$ cells (**A**). Neurospheres from the adult mouse SVZ were treated with Aph (5 μM) for 48 hr in the presence or absence of $A\beta_{42}$ (1 μM), and expression levels of nestin, Dlx-2, and GFAP were analyzed in total protein extracts (**B**). The percentages of cells immunopositive for the different lineage markers were scored by FACS analysis (**C–E**). Values are expressed as percentage of total cell population and are mean ± SEM of five different determinations from three independent experiments. *$P < 0.05$ (ANOVA followed by Tukey's post hoc test).

Lindsten et al., 2003). No change in apoptosis was detected in the Aph-treated group (Fig. 1D). These results indicated that NPC proliferation was effectively arrested in the G1/S phases, and under this condition no toxicity was observed. Western blot analysis showed an increased expression of p21 and $p27^{kip1}$ in neurospheres treated with Aph (Fig. 1D), whereas no changes were observed in the expression levels of Bax, a proapoptotic marker (Fig. 1D).

We then used nestin as a marker to quantify NPCs, Dlx-2 as an early marker of differentiation into the neuronal lineage, and PSA-NCAM as a marker of neuroblasts (Moraes et al., 2009). Neurospheres were analyzed by immunofluorescence staining (Fig. 2A), and the percentages of immunopositive cells for different markers were scored by FACS analysis. In the control group, neurospheres consisted mainly of nestin$^+$ cells (Fig. 2A,C). When $A\beta_{42}$ (1 μM) was added for 24 hr, NPCs showed a 20% increase in the percentage of nestin$^+$ cells (Fig. 2B,C) and accounted entirely for the population of cells also expressing Dlx-2 (nestin$^+$/Dlx-2$^+$; Fig. 2C). We also found an increased percentage of Dlx-2$^+$ cells that also stained for PSA-NCAM$^+$ in neurospheres treated with $A\beta_{42}$ (Fig. 2D). Dlx-2/PSA-NCAM-labeled neuroblasts were virtually absent in control cultures (Fig. 2D). In contrast to Aβ treatments, addition of Aph to cells cultures neither increased the number of nestin$^+$ cells (Fig. 2B,C) nor promoted the differentiation of nestin$^+$ cells toward the neuronal lineage (Fig. 2C,D) compared with the control group. Similarly to $A\beta_{42}$, Aph did not alter the percentage of cells expressing both nestin and GFAP (Fig. 2E).

Effect of $A\beta_{42}$ on DNA pol-β Expression and Role of DNA pol-β in NPCs in SVZ Neurospheres

The evidence that Aph did not mimic and did not potentiate the neurogenic effect of Aβ in SVZ neuro-



	Percentage of cells in phase		
	G_0/G_1	S	G_2/M
CTRL	73 ± 1.8	13 ± 2.1	14 ± 0.9
C shRNA	74 ± 2	12 ± 1.3	14 ± 1.2
shRNA	69 ± 1.6	19 ± 1.2*	12 ± 1.5
ddC	68 ± 1.5	18 ± 1*	14 ± 0.7

Fig. 3. DNA pol-β acted as a repressor of proliferation in suspended neurospheres. **A:** Confocal laser scanning microscopy of neurospheres. Cells were stained for nestin in green and for DNA pol β in red. **B:** Representative immunoblots of DNA pol-β in protein extracts from neurospheres exposed to 1 μM $A\beta_{42}$ for 24 hr. Cell cycle distribution profiles (**E**) and extent of apoptosis (**C**) in NPCs expressing either a DNA pol-β shRNA or a control shRNA encoding a scramble sequence (C-shRNA; **D**). Some neurospheres were treated with 2′,3′-dideoxycitidine (ddC; 100 μM), a selective inhibitor of DNA pol-β, for 24 hr. In E, $^*P < 0.05$ vs. the control (CTRL, C-shRNA). Values are mean ± SEM of three different determinations from five independent cultures. For each determination, 50,000 cells were analyzed by cytofluorimetric analysis.

spheres (Fig. 2B–D) indicated that cell cycle inhibition per se was not sufficient to account for Aβ-induced neurogenesis and that Aβ may exert a direct neurogenic effect on NPCs. Copani et al. (1999) reported that, in postmitotic neurons, $A\beta_{42}$ reactivated an aberrant cell cycle by engaging the base excision repair enzyme, DNA polymerase β (DNA pol-β), within a noncanonical pathway of neuronal DNA replication. Interestingly, DNA pol-β was shown to be required for proper neurogenesis during embryonic development (Sugo et al., 2000). We wondered whether $A\beta_{42}$-induced neurogenesis could be dependent on the activation of DNA pol-β in neurospheres. Virtually all the cells within the neurospheres constitutively expressed DNA pol-β (Fig. 3A,B), which was further increased (by approximately threefold) following exposure to $A\beta_{42}$ (1 μM) for 24 hr (Fig. 3B). To gain insight into the role of DNA pol-β in neurogenesis, we carried out shRNA-mediated silencing of the enzyme. The shRNA expression dramatically reduced the expression levels of DNA pol-β in total protein extract from NPCs (Fig. 3D). Twenty-four hours after shRNA had been delivered through lentivirus, neurospheres were analyzed for both cell cycle distribution and apoptosis. FACS analysis indicated that DNA pol-β knockdown by shRNA significantly increased the percentage of cells entering S phase, an effect that was shared by the pharmacological inhibitor of DNA pol-β ddC (Copeland et al., 1992; Fig. 3E). Basal levels of apoptosis were analyzed, but no statistically significant differences were observed between groups (Fig. 3C). Taken together, these data indicated that DNA pol-β could act as a repressor of proliferation of NPC neurospheres, consistent with previous data obtained with treatment of neurospheres with end-capped phosphothioate antisenses for DNA pol-β (1.5 μM for 16 hr; Copani et al., 2002). Both the antisense-induced knockdown of DNA pol-β and its pharmacological blockade with ddC induced a significantly

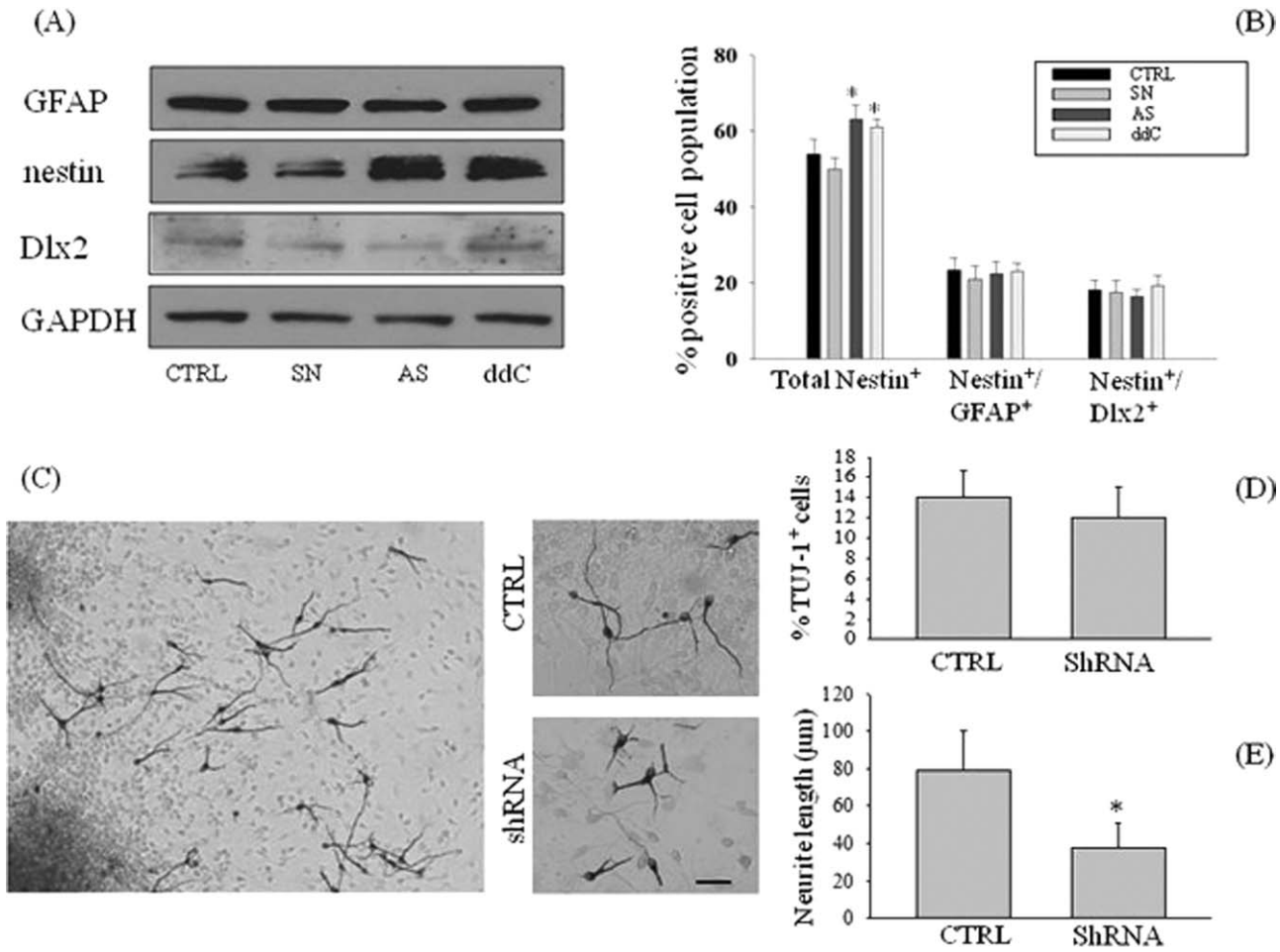


Fig. 4. Knockdown of DNA pol-β altered the differentiation of NPCs. **A:** Expression levels of GFAP, nestin, and Dlx-2 in total protein extracts from neurospheres treated with DNA pol-β antisenses (As) or senses (Sn) or treated with ddC (100 μM). GAPDH was used as a loading control. **B:** The percentage of total nestin$^+$ cells was increased in neurospheres treated with DNA pol-β antisenses (1.5 μM), but not the number of nestin$^+$ cells coexpressing GFAP or Dlx-2. *$P < 0.05$ vs. the controls. **C:** Neurospheres were plated on poly-L-ornithine- and laminin-coated dishes for 72 hr in growing medium in the absence of EGF. Immature neurons, in which DNA pol-β had been silenced with shRNA, were immunostained with TuJ-1, and the number of immunopositive cells (**D**) and neurite length (**E**) were compared with the controls (CTRL). For each determination, 20 randomly selected fields from three independent cultures were analyzed. Values are mean ± SEM of four different determinations from three independent experiments. In E, *$P < 0.05$ (ANOVA followed by Tukey's post hoc test) vs. the control group. Scale bar = 30 μm.

increase of nestin protein levels at 24 hr after treatment (Fig. 4A). This resulted from the increased number of early NPCs expressing nestin, but not GFAP or Dlx-2 (Fig. 4B), suggesting that DNA pol-β could restrain the proliferation of early NPCs in suspended neurospheres under basal conditions.

Neurospheres cultured onto laminin, in the absence of growth factors, consist of a mixture of precursor cells and differentiated neurons, astrocytes, and oligodendrocytes (Suslov et al., 2002). Twenty-four hours after the transfection with shRNA, neurospheres were plated onto poly-L-ornithine and laminin and allowed to differentiate for 3 days. We used an antibody against the β-tubulin III isoform (TuJ-1) to determine the percentage of immature neurons in culture. Although the silencing of DNA pol-β did not influence the percentage of TuJ-1$^+$ cells compared with controls (Fig. 4C,D), the assessment of neurite outgrowth revealed that neurons generated from shRNA-treated neurospheres showed a dramatic reduction of neurite length (38 ± 13 μm, n = 157) compared with the control group (79 ± 21 μm, n = 168; Fig. 4C,E).

Role of DNA pol-β in $A\beta_{42}$-Induced Neuronal Differentiation of SVZ Neuroshpheres

When $A\beta_{42}$ was added in suspended neurospheres in which DNA pol-β had been knocked down by antisenses, $A\beta_{42}$ treatment failed to increase the number of cells immunoreactive for both nestin and Dlx-2 (Fig.

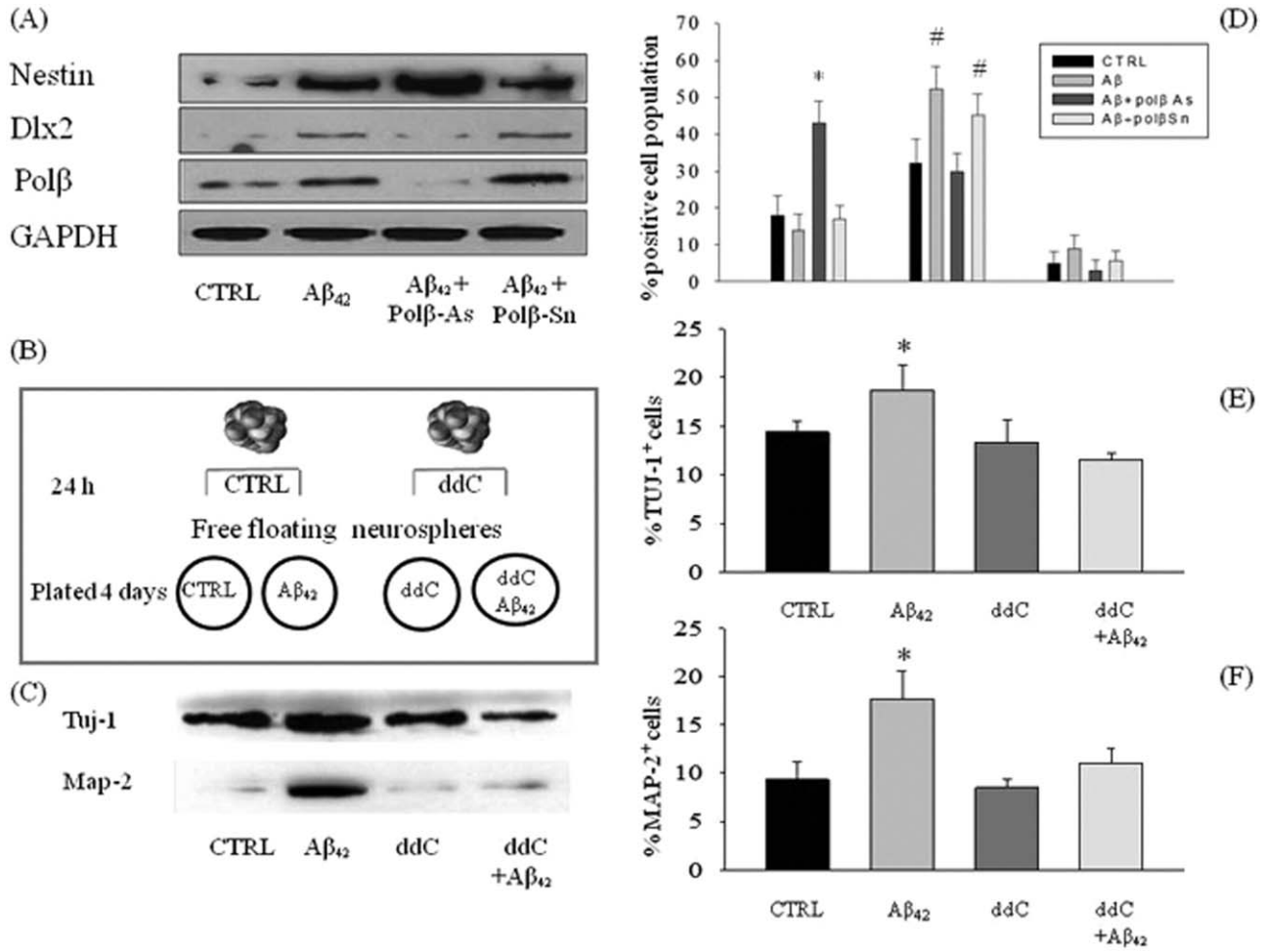


Fig. 5. DNA pol-β blockade inhibited neuronal differentiation in neurospheres exposed to $A\beta_{42}$. Cultures were treated with antisenses (pol β-As) or senses (pol β-Sn; 1.5 μM) for 16 hr before the addition of 1 μM $A\beta_{42}$. Representative immunoblots of nestin, Dlx-2, and DNA pol-β from neurospheres exposed to 1 μM of $A\beta_{42}$, after antisense or sense treatment, are shown in **A**. GAPDH was the loading control. **D:** pol β-As prevented the expression of Dlx-2 in neurospheres exposed to $A\beta_{42}$ for 24 hr. $^{*}P < 0.05$ vs. CTRL. $^{\#}P < 0.05$ vs. CTRL and Aβ-As. **B:** Suspended neurospheres were treated with ddC (100 μM) 24 hr before plating. Plated neurospheres were maintained in cultures for 4 days either in the absence or in the presence of 1 μM $A\beta_{42}$ and with or without ddC. **C:** Representative immunoblots of MAP-2 and TuJ-1 in protein extracts from neurospheres treated as described for B. The cell cycle inhibitor ddC prevented the increase in the total number of neurons (**F**) and neuroblasts (**E**) that was observed in neurospheres plated with $A\beta_{42}$. Data represent mean ± SEM of nine different determinations from three independent experiments. $^{*}P < 0.05$ vs. CTRL and $^{\#}P < 0.05$ vs. Aβ.

5A,D), and a parallel increase of nestin-only immunopositive cells was observed (Fig. 5D). These data indicated that DNA pol-β was engaged by $A\beta_{42}$ to promote the differentiation of early nestin^{+} NPCs toward the neuronal lineage in free-floating neurosphere cultures.

Next, neurospheres were plated onto laminin and allowed to differentiate for 4 days in the absence or presence of $A\beta_{42}$. Both TuJ-1^{+} cells and MAP-2^{+} cells, which correspond to more mature neurons, were found in control cultures (Fig. 5C,E,F). Exposure to $A\beta_{42}$ resulted into an increased number of both TuJ-1^{+} cells and MAP-2^{+} cells (Fig. 5C,E,F), indicating that $A\beta_{42}$ was able to promote the differentiation of NPCs into the neuronal lineage. Similarly to the shRNA-mediated silencing of DNA pol-β, pharmacological blockade of the enzyme with ddC (100 μM applied 24 hr before and during plating) did not affect the total number of TuJ-1^{+} cells in control cultures (Fig. 5C,E). However, the presence of ddC completely prevented the $A\beta_{42}$-induced increase of both TuJ-1^{+} and MAP-2^{+} cells (Fig. 5CE,F).

DISCUSSION

Enhanced neurogenesis has been reported in AD patients (Ziabreva et al., 2006) and in animal models of AD, including APP23 (Bondolfi et al., 2002), PDGF-APP_{Sw-Ind} (Jin et al., 2004a), and PDAPP mice

(Donovan et al., 2006), in all of which the human Aβ peptide is overproduced. Jin and colleagues (2004b) suggested that increased neurogenesis could be a compensatory response to disease manifestation, whereas López-Toledano and Shelanski (2007) proposed that Aβ itself drives neurogenesis in the early phases of AD, leading to the exhaustion of the NPC pool available for brain repair later in the neurodegenerative process of the disease. Our early work demonstrated that synthetic human $A\beta_{42}$, in the conformation state that is usually toxic for adult neurons, promoted instead the differentiation of NPCs of the adult mouse SVZ toward the neuronal phenotype (Calafiore et al., 2006). The mechanisms underlying this phenomenon are unknown. In the present study, our data indicate that synthetic oligomers of $A\beta_{42}$ drive the differentiation of nestin$^+$ NPCs into MAP-2$^+$ neurons by engaging the DNA repair enzyme DNA pol-β. High levels of DNA pol-β are found in the rat brain at embryonic ages (Nowak et al., 1990).

Neuronal differentiation may arise from genomic rearrangement factors that generate a certain type of DNA damage that is repairable by DNA pol-β (Sugo et al., 2004). However, the specific role of DNA pol-β in Aβ-induced neurogenesis has not been studied. We have shown that DNA pol-β acted to arrest the proliferation of a subset of nestin$^+$ progenitor cells, allowing them to acquire the early marker of the neuronal lineage, Dlx-2, in response to $A\beta_{42}$. This effect appeared to be specific for DNA pol-β, because a generic arrest of NPC proliferation by Aph, both in the absence and in the presence of $A\beta_{42}$, did not result in neuronal differentiation. Although DNA pol-β was expressed in virtually all NPCs, it appeared that its prodifferentiation effects were restricted to a population of progenitors committed to the neuronal lineage by $A\beta_{42}$. It is well known that NPCs are heterogeneous, and this heterogeneity is particularly likely in the NPC population in cultured neurospheres. The evidence that DNA pol-β per se was required for the maturation of TuJ-1$^+$ cells suggested that the enzyme could serve multiple functions in cells belonging to the neuronal lineage. It was intriguing that DNA pol-β appeared to control proliferation and differentiation of nestin$^+$ progenitors, but it was not required for cell survival. A hypothetic model of the DNA pol-β involvement in Aβ-induced neurogenesis is presented in Figure 6. This model is based on results from this study as well as those from our previous study (Calafiore et al., 2006). Our findings strengthen the molecular link between the function of DNA poly-β and the induction of neural differentiation.

DNA pol-β might be critically involved in the differentiation of neural progenitors for several reasons. First, DNA pol-β might be part of an endogenous "checkpoint system" aimed at regulating the quantity and quality of nestin$^+$ progenitors via cell cycle arrest. Second, DNA pol-β might function to maintain the integrity of genes (e.g., Dlx-2) to be expressed in certain progenitors (e.g., nestin$^+$/Dlx-2$^+$ cells), thus coordinating proliferation arrest with differentiation. Third, DNA

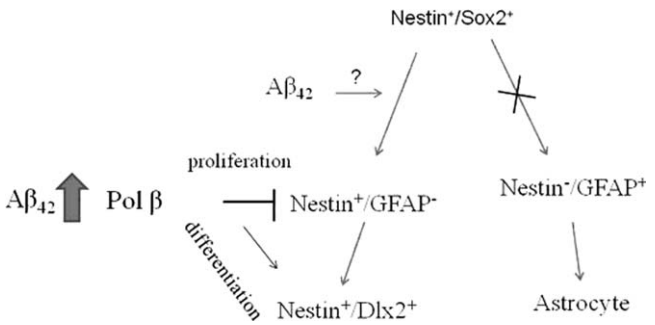


Fig. 6. Model depicting the role for DNA pol-β in the neurogenic effect of Aβ. This hypothesis of neuronal differentiation driven by Aβ is based on the results from the present study and from Calafiore et al. (2006). $A\beta_{42}$ arrests the proliferation of a subpopulation of nestin$^+$ cells via the induction of DNA pol-β, thus allowing for their differentiation toward the neuronal phenotype. $A\beta_{42}$ also promotes the appearance of nestin immunoreactivity in nestin$^-$/Sox-2$^+$ cells. The precise mechanisms underlying this effect remain to be determined.

pol-β might be required to repair oxidative base damage occurring in neuronal types actively undergoing axon pathfinding (Sugo et al., 2004). Consistently with this notion, our results indicated that the neurite outgrowth of Tuj-1$^+$ cells was dependent on the presence of DNA pol-β. Although it appeared that constitutive DNA pol-β participated in neurogenesis, our results indicated that an increased expression of the enzyme per se, in the absence of the concomitant stimulation by $A\beta_{42}$, would not be sufficient to promote neuronal differentiation fully.

Our findings reveal a novel role of DNA pol-β in $A\beta_{42}$-induced neurogenesis and also indentify DNA pol-β as a key mechanistic link between the neurogenic effect of $A\beta_{42}$ on NPCs and the proapoptotic effect of $A\beta_{42}$ on mature neurons. Because DNA pol-β is also involved in Aβ-mediated apoptosis of mature neurons (Copani et al., 2006), Aβ-induced neurons might undergo apoptosis once they reach the postmitotic state. Thus, it is likely that Aβ-induced neurogenesis in AD is fated to be unproductive. Nevertheless, DNA pol-β could participate to the attempted neurogenesis in AD and under many other neuropathological conditions, such as brain ischemia, in which the enzyme activity is expected to be increased as a result of the concomitant DNA damage that has to be repaired.

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