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Original article

Basic fibroblast growth factor stimulates the proliferation and differentiation of neural stem cells in neonatal rats after ischemic brain injury

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Abstract

A little is known about the proliferation and fate of neural stem cells in the subventricular zone (SVZ) after cerebral ischemia. However, how endogenous neural stem cells are activated in the premature brain is not clear, although basic fibroblast growth factor (bFGF) is important in neurogenesis. To investigate the effect of bFGF on the proliferation and differentiation of neural stem cells after brain ischemia, we observed cellular changes in the subventricular zone (SVZ) of 3-day-old rats (approximately equivalent to premature infants) using immunofluorescence assays, Western blot analysis, and real-time quantitative PCR methods. The bilateral common carotid artery (BCCA) was occluded in 108 animals, then half received bFGF 10 ng/g. Besides, 54 rats without ischemia as normal control. Proliferating cells were labeled by bromodeoxyuridine (BrdU) through intraperitoneal injection in a pulsed or a cumulative protocol. Rats were killed at 4, 7, and 14 days after ischemic injury. The number of proliferating cells in the SVZ in bFGF-treated rats was higher than that in untreated rats; bFGF also promoted neural stem cell differentiation into neurons, astrocytes, and oligodendrocytes. Western blot analysis and real-time quantitative PCR assays confirmed these results. We suggest that bFGF promotes the repair of ischemia brain injury through increasing the proliferation of neural stem cells and their differentiation into neurons, astrocytes, and oligodendrocytes.

Keywords: Rat; Subventricular zone; Bromodeoxyuridine; Neurogenesis; Brain ischemia

1. Introduction

Ischemic brain injury in premature infants is a problem of enormous importance [1]. Despite a large number of studies with promising neuroprotective agents, no clinically successful strategy for neuroprotection has emerged [2]. Furthermore, therapeutic hypothermia has been used for newborns with hypoxic ischemic encephalopathy, but further trials are required [3]. Ideally, all cells or tissues lost as a result of injury would be readily regenerated.

The immature neonatal brain maintains a similar or perhaps even greater potential for neuronal repair after hypoxic–ischemic injury than does the adult brain. In 2001, Levison et al. found that hypoxic–ischemic injury results in the sustained depletion of neural stem cells [4]. Moreover, cells within the subventricular zone (SVZ) are particularly sensitive to insults [5]. Thus, the repair of neonatal brain injury through activating the endogenous neural stem cells in SVZ has received much attention.

Basic fibroblast growth factor (bFGF) belongs to the family of polypeptide growth factors that are involved in embryonic development and adult tissue homeostasis. Numerous studies of bFGF suggest that it plays a

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ubiquitous and life-long role in neurogenesis [6,7] in adult rat. Moreover, bFGF enhances functional recovery in an adult rat model of focal ischemia [8]. Therefore, it may have value in treating ischemic brain injury in premature infants. Therefore, we studied the effects of bFGF on the proliferation and differentiation of neural stem cells in neonatal rats after induced ischemic brain injury.

2. Materials and methods

2.1. General preparation

We obtained 162 postnatal day 3 (P3) Sprague–Dawley rats weighing between 8.2 and 10.5 g from the Experimental Animal Center, Shanghai Medical College, Fudan University, China. The bilateral common carotid artery (BCCA) was occluded in 108 animals, and then the rats were divided into a treatment group of 54 rats receiving bFGF and a control group of 54 untreated rats. Besides, 54 rats were divided into the sham operation group, whose BCCA was not occluded (Fig. 1). To minimize the effect of the litter-specific response, rats in the same litter were divided into the above-mentioned three groups randomly. From each group, 18 animals were killed at each of three time points, 4, 7, and 14 days after ischemia was induced.

All efforts were made to minimize both the suffering and the number of animals used. All procedures were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals, and have been approved by the Institutional Care of Experimental Animals Committee of Fudan University.

2.2. Animal model

Animals were anesthetized with anhydrous diethyl ether. The criterion of the anaesthesia depth is that rats have normal respiratory rhythm and no response to stimulus. Rectal temperature was maintained at 37 °C, plus or minus 1 °C, throughout the procedure using a feedback-regulated water heating system. As described by Cai et al. [9], after the rats were fully anesthetized, a midline neck incision was made and the BCCA was identified. The artery was separated from the vagus nerve, ligated using 6-0 silk, and the incision was sutured. The BCCA of rats in the sham operation group was not occluded. Following operation, rats were injected with bFGF or normal saline. Briefly, a stainless steel needle was stereotaxically implanted into the left lateral cerebral ventricle (stereo coordinates: A 3.8 mm for anterior–posterior; L 2 mm for lateral; H 3.2 mm for dorsoventral) [10]. The treatment rats were then injected with 10 ng/g of bFGF (0.1 mg/mL), the control rats and the sham rats were injected with normal saline (2 μL). The animals were then returned to dam after a 1 h recovery period in an incubator at 37 °C.

2.3. Bromodeoxyuridine (BrdU) labeling

BrdU (Sigma, USA), the thymidine analog that is incorporated into the DNA of dividing cells during S-phase, was used to label proliferating cells [11]. Two-thirds of all rats received BrdU. To assess the rate which new proliferative cells were produced, we injected half the rats with BrdU (50 mg/kg) intraperitoneally every 4 h for 12 h on the day before the animals was killed (pulse labeling). To assess the total population of prolif-

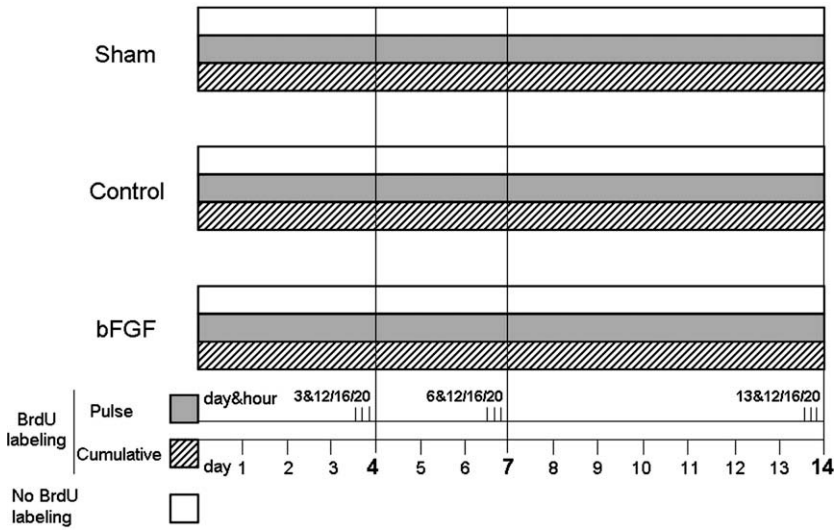

Fig. 1. Illustration of study design. All rats were divided into sham, control, and bFGF groups randomly, and were killed at days 4, 7, and 14 after operation, respectively. Every group was divided into pulse BrdU labeling, cumulative BrdU labeling and no BrdU labeling subgroups. There are 6 rats in every subgroup at each time point.

erative cells in the brain that were produced since the ischemic injury, we injected the remaining half of the rats with BrdU (50 mg/kg) intraperitoneally once daily from operation induction to euthanasia (cumulative labeling) (Fig. 1). Thus, 36 rats in each group received BrdU administration, half of which were pulse labeled and half cumulatively labeled. Twelve rats from each group were euthanized on each time point.

2.4. Histology

Fifteen minutes before they were killed at the above-mentioned time points, the BrdU-labeled rats were transcardially perfused with heparinized saline and then by 4% paraformaldehyde. Their brains were removed, the SVZ were dissected, and then immersion-fixed in 4% paraformaldehyde for 24 h at 4 °C. The SVZ from the animals was obtained according to the established methods [12]. Briefly, brain tissue between chiasm opticum and hippocampus was dissected firstly, and then tissue outside lateral ventricle and corpus callosum was removed (Fig 2). The next day, the SVZ were dehydrated through graded sucrose (10%, 15%, and 30% for 24 h at each grade) at 4 °C and then embedded in embedding medium OCT, preserved at −80 °C. Frozen sections of SVZ, 10 μm-thick, were used for the immunofluorescence assays.

The remaining rats, which were not administrated BrdU, were anesthetized with anhydrous diethyl ether and killed at the above-mentioned time points. Their brains were removed, and then SVZ were dissected, rinsed with 0.1 M PBS, preserved in liquid nitrogen.

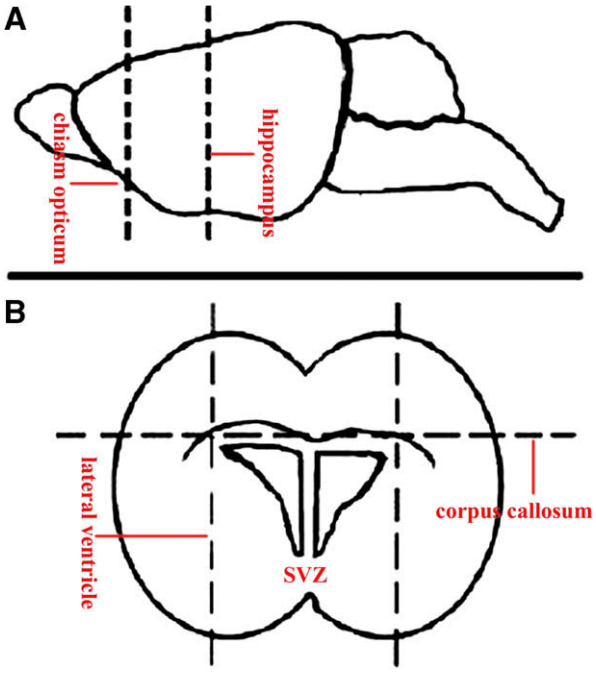

Fig. 2. Dissection of the SVZ.

All apparatus we used were free RNAase. Within 6 rats in each group at each time point, 3 were used for Western blot analysis and 3 to observe gene expression with real-time PCR assays.

2.5. Immunofluorescence assays

Using antibodies for BrdU, Nestin, NeuN, GFAP, and NG2, we assessed the proliferation and differentiation of neural stem cells in the SVZ with immunofluorescence assays. Cells were labeled with Anti-BrdU antibody. Additionally, cells were labeled with antibodies for Nestin to identify neural stem cells, for NeuN to identify neurons, for GFAP to identify astrocytes, and for NG2 to identify oligodendrocytes. All methods followed published protocols [13,14].

For the immunofluorescence assays, sections from the SVZ were washed (0.1 M Tris, pH 7.6, 15 min), denatured (2 N HCl, 37 °C, 30 min), rinsed (0.1 M PBS, 10 min), incubated with 1% H_2O_2 in 0.1 M Tris for 30 min, rinsed, blocked (10% normal goat serum, 37 °C, 30 min), and incubated with a rat monoclonal anti-BrdU (1:200, 4 °C, over night, LAB VISION, USA). Sections were rinsed (0.1 M PBS, 10 min), incubated with tetraethyl rhodamine isothiocyanate (TRITC)-conjugated goat anti-rat IgG (1:100, room temperature, 2 h, Jackson ImmunoResearch Laboratories Inc., USA), rinsed, then incubated with the other mouse anti-rat monoclonal 1^0 antibody [4 °C, overnight, anti-Nestin (1:200, R&D, USA), anti-NeuN (1:200, Chemicon, USA), anti-GFAP (1:100, Neuromics, UK), and anti-NG2 (1:200, Chemicon, USA), respectively]. All sections were washed and incubated with 2^0 antibodies, and rinsed, mounted, dried, and coverslipped using DAPI. All 2^0 antibodies were fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgM (1:100, room temperature, 2 h, Jackson ImmunoResearch Laboratories Inc., USA).

Immunofluorescence staining was visualized by fluorescence microscopy (Leica, Germany). Cell proliferation was quantified using stereologic counting of BrdU-positive cells in the SVZ at the above-mentioned time points in each group ($n = 6$). Every 4th section in a series of 10 μm-thick sections through the SVZ was sampled, following the methods of Plane et al. [15]. BrdU immunofluorescence assays were performed as described above; equidistant sections (3/brain) were used for stereologic counting to assess cell proliferation in the SVZ. BrdU-positive cells in the SVZ were counted in a randomly selected section for each brain; this section was used to determine cell counting frame size, number of samples/section, and spacing of sample sites. All counts were performed using a 200× objective lens on a Leica fluorescence microscope and the StereoInvestigator v.5.0 computer program (MicroBrightField, Colchester, VT). The total number of cells/region was estimated using the Optical Fractionator technique [16].

2.6. Western blot analysis

Frozen tissues of SVZ were cut into small pieces and homogenized in 0.5 mL of RIPA buffer (150 mmol/L NaCl, 1% N-40, 0.5% deoxycholate, 0.1% sodium dodecylsulfate, 50 mmol/L Tris–hydrochloric acid, 2 mmol/L phenylmethylsulfonyl fluoride, pH 7.4), then transferred into small tubes and rotated at 4 °C overnight. Solubilized protein was collected after centrifugation at 10,000*g* for 30 min. The supernatant from each culture was collected, and protein concentrations were quantified with the Enhanced BCA Protein Assay Kit (Beyotime Biotechnology, Haimen, China).

To detect Nestin, GFAP, NeuN, and NG2 protein levels, tissue protein lysate from each group of rats were subjected to SDS–PAGE analysis with 10% (wt/vol) acrylamide gel and electrotransferred onto a PVDF membrane (Millipore Corporation). The nonspecific proteins on membranes were blocked in 5% skim milk powder in PBS 0.1% Tween-20 2 h at room temperature. Immunoblotting was performed using 2 μg/mL of mouse anti-rat Nestin monoclonal antibody (R&D, USA), or 1 μg/mL of mouse anti-rat NeuN mAbs (Chemicon, USA), or 10 μg/mL of mouse anti-rat GFAP mAbs (Neuromics, England), or 1 μg/mL of mouse anti-rat NG2 mAbs (Chemicon, USA), respectively. The membranes were then incubated with the appropriate secondary horseradish peroxidase-conjugated anti-mouse IgG antibodies at a 1:2500 dilution (Jackson ImmunoResearch Laboratories Inc., USA). Immunoreactive proteins were then visualized using ECL plus a Western blotting detection system (Beyotime Biotechnology, Haimen, China).

2.7. Reverse transcription real-time quantitative PCR assays

We extracted total RNA from the frozen tissues of SVZ using TRIzol reagent following the manufacturer's suggested protocol (Invitrogen, USA). RNA concentrations were measured spectrophotometrically in a GenQuant RNA/DNA calculator (Pharmacia Biotech, UK). For reverse transcription (RT), we prepared 2 μg of total RNA and random primer 1 μL for each sample, then added RNase-free water to 15 μL and kept at 70 °C for 5 min before being cooled on ice. We then added 5× MMLV RT buffer 5 μL, dNTP 1.25 μL, RNase inhibitor (Toyobo Co., Ltd., Osaka, Japan) 25 U (0.6 μL), MMLV Reverse transcriptase (Promega, USA) 200 U (1 μL) and 2.15 μL RNase-free water. Reverse transcription of 2 μg RNA for cDNA synthesis was performed in a total volume of 25 μL. Reverse transcription reaction was carried out in the Thermal Cyclers PTC-150 (MJ Research, USA) using a program with 40 RT cycles at 25 °C for 10 min, followed by 60 min at 37 °C and 5 min at 95 °C, then quenched at 4 °C. After the reaction was completed, samples were stored at −20 °C until real-time PCR.

Real-time quantitative PCR was conducted as described in the instruction manual for Real-time PCR Master Mix (Toyobo Co., Ltd., Osaka, Japan). cDNA samples 1 μL, and gene-specific primers (sense primer 1 μL, anti-sense primer 1 μL) were added to SYBR Green PCR Master Mix 25 μL, and then ddH_2O was added to 50 μL total volume. PCR amplification (1 cycle at 95 °C for 20 min, 40 cycles at 95 °C for 30 s, 52 °C for 30 s, 72 °C for 40 s) was performed in the Eppendorf Mastercycler®ep realplex (Eppendorf, Germany). For each RT, real-time PCR was conducted three times in duplicate using each of the cDNA samples. The amplified transcripts were quantified with the comparative CT method using standard curves. The primers were designed using the Primer Express software (Primer premier 5.0) based on the GenBank Accession Nos., and the sequences were as Table 1. Actin-β as the internal control.

2.8. Statistic methods

All values are presented as means and standard deviations. One-way analysis of variance (ANOVA) was used to examine differences between the control group and the treatment group at each time point. The LSD procedure was used for pairwise comparisons. Alpha set at 0.05, and all tests were two-tailed. All analyses were performed using SPSS 12.0.

3. Results

3.1. Pathological changes following BCCA

Haematoxylin–eosin staining was used to observe the histological change following BCCA. There were no changes in the sham operation group. The pathological changes at day 4 were significant and then relieve from the 7th day after the operation in the rats of control

Table 1
Primer sequence of 5 genes used for quantitative real-time PCR

Gene name (official symbol)	Accession No. (GenBank)	Primer sequence (5′–3′)
Nestin (Nes)	NM_012987	F: GGAGCAGGAGAAGCAAGGTC R: GGGTCCAGAAAGCCAAGAG
Tubulin-β 3 (Tubb3)	NM_012987	F: CCCCAGCTTACCTTCCTACC R: GAGGACAGAGCCAAGTAAAC
Glial fibrillary acidic protein (GFAP)	NM_017009	F: AGAAAACCGCATCACCATTC R: GCATCTCCACCGTCTTTACC
Chondroitin sulfate Proteoglycan 4 (Cspg4)	NM_031022	F: TGCAGCACAAAAGGATCTATG R: AGCACTGCCTCCTGGACTAC
Actin-β (Actb)	NM_031144	F: TGACAGGATGCAGAAGGAGA R: TAGAGCCACCAATCCACACA

Fig. 3. Pathological changes in the SVZ in rats at day 4 after operation with Haematoxylin–eosin staining. There were cerebral edema widely, neuronal cells necrosis and focal cerebromalacia formation in control groups. These damages in treatment group are mild than those in control group and there are no changes in the sham operation group.

and treatment group. Moreover, the histological changes were severe in the rat's brain of control group. There were cerebral edema widely, neuronal cells necrosis, and focal cerebromalacia formation. These damages in treatment group are mild than those in control group (Fig. 3).

3.2. Affects of bFGF on the proliferation and differentiation of neural stem cells

Pulse BrdU labeling was used to determine changes in the number of proliferating cells over time. BrdU-positive cells were detected in the SVZ in all pulsed-labeled rats. The number of BrdU-positive cells was highest at 7 days in all groups. Treatment with bFGF significantly increased the number of BrdU-positive cells in the SVZ at all three time points ($P < 0.01$) (Figs. 4A and 5A). These data indicate that cells proliferate soon after the onset of ischemia and that early administration of bFGF may promote cell proliferation in the neonatal rat brain.

To label proliferative cells, we used cumulative BrdU labeling. The number of BrdU-positive cells in the SVZ gradually increased over time in control and treatment groups, suggesting that cells proliferate continually for 14 days after the onset of ischemia. Rats treated with bFGF had significantly more BrdU-positive cells in the SVZ than did controls at all three time points ($P < 0.01$). These data indicate that bFGF can promote cell proliferation in the neonatal rat brain during 14 days after the onset of ischemia.

To determine whether the BrdU-labeled cells expressed phenotypic neuronal features, we double-labeled frozen sections with antibodies against BrdU and against Nestin, a marker for neural stem cells; neuron-specific nuclear protein (NeuN), a marker for mature neurons; glial fibrillary acidic protein (GFAP), a marker for astrocytes; and chondroitin sulfate proteoglycan 4 (NG2), a marker for oligodendrocytes. The number of BrdU-positive/Nestin-positive cells was higher at all three time points in the bFGF-treated rats. Pulse BrdU labeling showed that the number of BrdU-positive/Nestin-positive cells in the control group peaked at 7 days, and the number of BrdU-positive/Nestin-positive cells in the bFGF-treated group was higher than that of the control group (Figs. 4B and 5B). These data suggest that neural stem cells mainly proliferated soon after the onset of ischemia. The number of BrdU-positive/NeuN positive cells, BrdU-positive/GFAP-positive cells, and BrdU-positive/NG2-positive cells peaked at 14 days. Treatment with bFGF significantly enhanced the number of these three group cells at all three time points. Most BrdU-positive cells expressed NeuN in the bFGF-treated rats (Figs. 4C–E and 5C–E).

3.3. Affects of bFGF on neuroprotein expressions

Western blot analysis also showed that Nestin, NeuN, GFAP, and NG2 protein expression were highest in the treated group at all time points compared with those in control group and sham operation group ($P < 0.01$). The expression of Nestin peaked on day 7, and the expression of NeuN, GFAP, and NG2 peaked on day 14 (Fig. 6).

3.4. Affects of bFGF on Nestin mRNA expression

Nestin mRNA expression was highest in bFGF-treated rats than in controls and in sham operation groups ($P < 0.01$). The number peaked at day 7. Tubulin-β 3, GFAP and Cspg4 mRNA expressions were highest in bFGF-treated rats than in controls and in sham operation groups, respectively ($P < 0.01$). The number peaked at day 14. Actin-β mRNA expression did not differ among groups (Table 2).

4. Discussion

Neuronal loss is a major feature of numerous brain diseases, including ischemic brain injury in premature infants. Although cell replacement therapy has recently been advanced, little is known about how neurogenesis is regulated in the repair neuronal deficiencies. Neural stem cells have the potential for self-renewal; can proliferate and differentiate into neurons, astrocytes, and oligodendrocytes under certain conditions; and may be involved in restoring neuronal functioning [17,18]. Interestingly, this particular new form of structural brain plasticity seems to be specific to discrete brain regions;

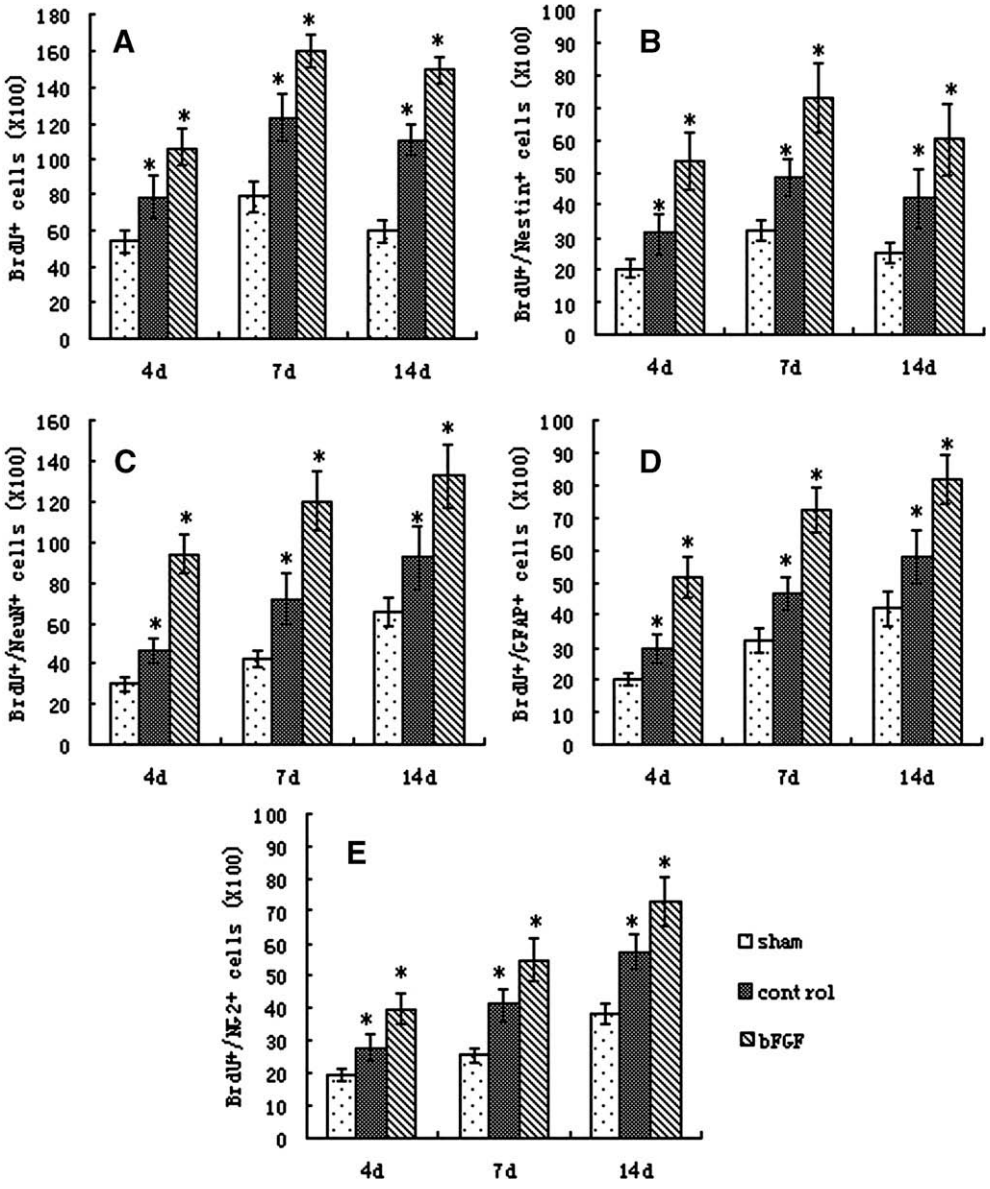

Fig. 4. Quantitative analysis of different newborn cells in the SVZ after brain ischemia. The number of BrdU+ and BrdU+/Nestin+ cells increased and peaked at day 7 after BCCA occlusion. Administration of bFGF significantly increased the number of both types of cells (A, BrdU+ cells; B, BrdU+/Nestin+ cells). The number of BrdU+/NeuN+, BrdU+/GFAP+, and BrdU+/NG2+ cells increased and peaked at day 14. Administration of bFGF also significantly increased the number of these three type cells (C, BrdU+/NeuN+ cells; D, BrdU+/GFAP+ cells; E, BrdU+/NG2+ cells). Values are means and SDs. Values were compared with ANOVA, $^{*}P < 0.01$.

most investigations concern the SVZ and the dentate gyrus of the hippocampal formation.

The SVZ generates the majority of the neurons and glial cells in the mammalian CNS [19]. Over the past decade, the cells of the SVZ have been studied intensely because they harbor neural stem cells. The SVZ is composed of a mosaic of immature cell types that includes multipotential, bipotential, and unipotential stem cells, as well as progenitor cells at different stages of lineage restriction [16,20]. During the last third of fetal development, the SVZ expands tremendously, and this expansion continues into the perinatal period [21,22]. Furthermore, the proliferative and differentiating activities of the neural stem cells are not stable; rather they are dynamically regulated by various humoral and adhesive factors under physiological and pathophysiological conditions [23]. Thus, understanding the factors regulating neural stem cell activity may contribute not only to understanding neural ontogeny but also to developing new therapies against neural death. By studying the proliferation and differentiation of neural stem cells in the SVZ after brain ischemia and the administration of exogenous bFGF, we hoped to determine whether bFGF treatment might ameliorate ischemic brain injury in neonates.

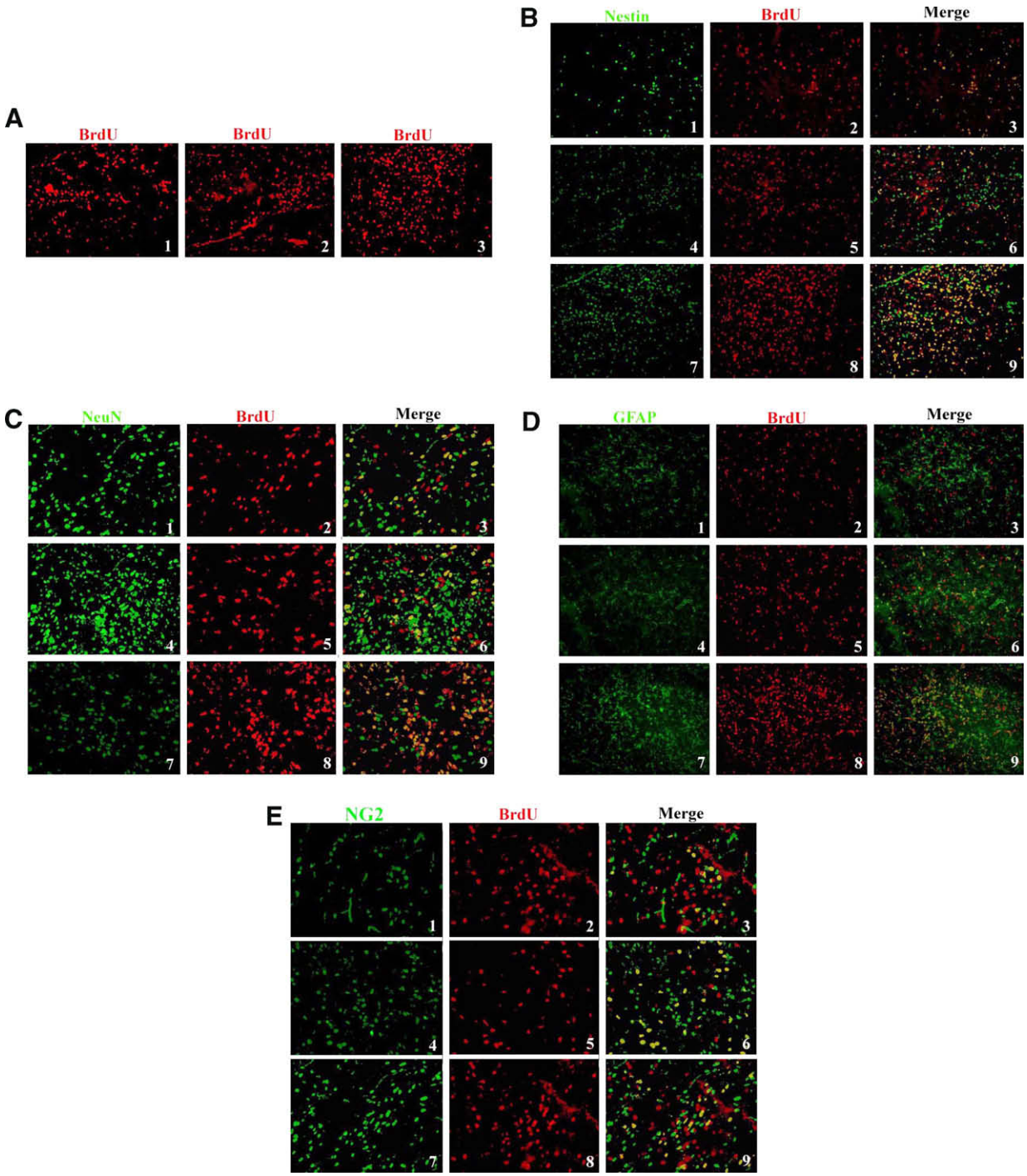

Fig. 5. Immunofluorescence staining in the SVZ on frozen sections in rats. (A) Pulse BrdU labeling in rats at day 7 (1, sham operation; 2, controls; 3, treatment). (B) BrdU and Nestin double labeling in rats at day 7 (1–3, sham operation; 4–6, controls; 7–9, treatment). (C–E) BrdU and NeuN, GFAP, NG2 double labeling in rats at day 14, respectively (1–3, sham operation; 4–6, controls; 7–9, treatment).

BrdU, an analog of thymidine, is incorporated into the DNA during the S-phase and has been used to investigate cell proliferation. Our BrdU labeling data revealed significant increases in the number of BrdU-labeled cells in the SVZ after bilateral common carotid artery occlusion. The increased proliferation persisted at least 14 days after the onset of ischemia and peaked at day 7.

Transient increases in proliferating progenitor cells in the injured brain indicate that there is a window for endogenous brain plasticity. Therefore, manipulating progenitor cell proliferation, migration, and differentiation within this window could enhance brain plasticity. Our experimental data support this premise. The number of BrdU-positive cells was higher in rats treated with bFGF than in controls, suggesting that

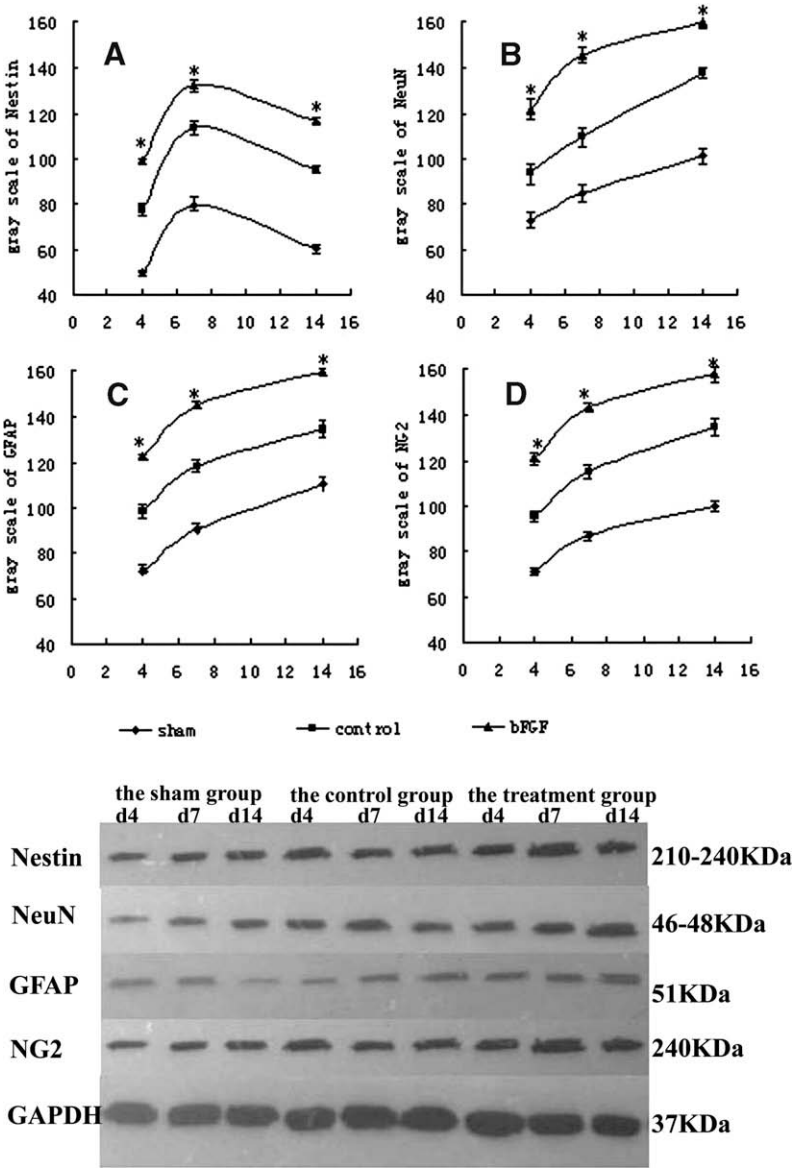

Fig. 6. Nestin, GFAP, NeuN, and NG2 protein levels were detected by Western blot analysis. Nestin, NeuN, GFAP, and NG2 protein expression were highest in treated group at each time point. Protein levels are expressed as means and SDs. Values were compared with ANOVA, $^{*}P < 0.01$.

bFGF helps promote cell proliferation in the SVZ after ischemic injury.

Although ischemia induces cortical and subcortical gliosis, cell proliferation in the SVZ has not been fully

Table 2
Changes in the expression of Nestin, Tubb3, GFAP, and Cspg4 mRNA over time in SVZ of neonatal rats following bilateral common carotid artery occluded (log copies (SD)/2 μg total RNA, $P < 0.01$)

Time	Nestin mRNA			Tubb3 mRNA		
	Sham	Control	Treatment	Sham	Control	Treatment
Day 4	2.92 (0.31)	3.85 (0.41)	5.24 (0.56)	1.76 (0.18)	1.83 (0.19)	2.67 (0.46)
Day 7	3.41 (0.38)	4.11 (0.53)	7.45 (0.87)	2.03 (0.22)	2.41 (0.32)	4.35 (0.47)
Day 14	2.78 (0.29)	3.62 (0.40)	6.76 (0.79)	3.12 (0.35)	4.64 (0.51)	6.86 (0.72)
	GFAP mRNA			Cspg4 mRNA		
Day 4	2.47 (0.32)	3.44 (0.45)	6.24 (0.86)	1.77 (0.21)	2.84 (0.47)	5.61 (0.76)
Day 7	3.51 (0.38)	5.11 (0.62)	8.45 (0.97)	2.23 (0.30)	4.41 (0.64)	7.79 (0.85)
Day 14	3.77 (0.41)	6.62 (0.84)	9.76 (1.29)	3.12 (0.37)	5.72 (0.67)	9.23 (1.13)

explored. We found that occluding the BCCA significantly increased the number of BrdU-labeled cells in the SVZ, suggesting that cerebral ischemia induces cell proliferation. The ventricular zone and the SVZ give rise to most neurons and glial cells in the forebrain during the embryonic period [24]. To investigate whether the BrdU-labeled cells expressed phenotypic neuronal features, we used different markers (Nestin for neural stem cells, NeuN for mature neurons, GFAP for astrocytes, and NG2 for oligodentrocytes) to double-label the sections. Within 7 days after ischemia, most of BrdU-positive cells were Nestin-positive, and the number of these cells increased over time, suggesting that ischemia stimulates the self-renewal of neural stem cells. The number of Nestin-positive/BrdU-positive cells after bFGF treatment also increased. Western blot analysis and real-time quantitative PCR also showed that Nestin expression was higher in bFGF-treated rats than in controls. These data confirm that bFGF promoted the proliferation of neural stem cells.

BrdU-positive cells expressing NeuN, a mature neuron marker, and GFAP, an astrocyte marker, also increased in the SVZ affected by ischemic injury. These results indicate that brain ischemia stimulated neural stem cells to differentiate into neurons and astrocytes, which might have a self-repair role in ischemia injury. Treatment with bFGF increased the expression of NeuN and GFAP in BrdU-positive cells in the SVZ over that in controls, and Western blot results confirmed this increase. Tubulin-β 3 expression characterizes the differentiating neuron. Real-time quantitative PCR also showed that Tubulin-β 3 and GFAP mRNA expression were higher in bFGF-treated rats than in controls. From these data, we conclude that bFGF treatment increases the differentiation of neural stem cells into neurons and astrocytes and thus promotes the recovery of ischemic brain injury in this way.

In neonatal animal models, oligodendrocytes have been susceptible to hypoxic–ischemic injury [25], a finding consistent with the effects of ischemic brain injury in premature infants. Chondroitin sulfate proteoglycan 4 expression characterizes the differentiating oligodendrocyte. We found that the number of oligodendrocytes (BrdU-positive/NG2-positive cells) increased after ischemic injury, and that bFGF treatment further increased this number. Western blot analysis and real-time quantitative PCR also confirmed the results. These data indicated that bFGF helps repair injured oligodendrocytes and that it can promote the recovery of ischemic brain injury by activating neural stem cells to differentiate into oligodendrocytes.

In summary, we studied the role of bFGF on the proliferation and differentiation of neural stem cells in neonatal rats after ischemic brain injury. Treatment with bFGF not only increased proliferation of neural stem cells, but also stimulated these cells to differentiate into neurons, astrocytes, and oligodendrocytes after ischemic injury. These data suggest that bFGF helps repair neonatal ischemic brain injury in neonatal rats.

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