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Moderate traumatic brain injury promotes neural precursor proliferation without increasing neurogenesis in the adult hippocampus

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ABSTRACT

Traumatic brain injury (TBI) promotes neural stem/progenitor cell (NSC) proliferation in the adult hippocampus; however, it remains inconclusive whether proliferation of these cells results in newly generated mature neurons, leading to increased neurogenesis. When we traced the fates of proliferating cells labeled with bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU) we found that the number of BrdU-positive cells increased in the hippocampus of TBI mice compared to the sham control. However, double immunostaining to distinguish their cell types showed that most of these cells were glia, and that only a small subpopulation is newborn granular neurons. There was no significant difference with respect to neurogenesis in the adult hippocampus between the injured and the control mice. These results indicate that TBI promotes cell proliferation including astrocyte activation and NSC proliferation. Nevertheless, the majority of the BrdU-positive cells are glia. The neurogenesis is not increased by TBI. These data suggest that TBI activates through promotion of NSC proliferation an innate repair and/or plasticity mechanism in the brain. However, additional intervention is required to increase neurogenesis for successfully repairing the damaged brain following TBI.

Introduction

Traumatic brain injury (TBI) is a serious public health problem in the United States (Cicerone et al., 2005; McCarthy et al., 2005; Prigatano, 2005; Salmond and Sahakian, 2005; Stiles et al., 2005; Vakil, 2005). It represents a complex injury with a broad spectrum of symptoms and disabilities. Effective treatment options are nonexistent. Recent research has identified neural stem/progenitor cells (NSCs) in the adult mammalian hippocampus that can support neurogenesis throughout life, as demonstrated in rodents and primates, including humans (Cameron and McKay, 2001; Eriksson et al., 1998a,b; Kornack and Rakic, 1999; Kuhn et al., 1996; Leuner et al., 2007). Currently the consensus among researchers in the field is that throughout adulthood, NSCs in the subgranular zone (SGZ) of the hippocampal dentate gyrus (HDG) continuously generate new neurons (Kempermann and Gage, 2000; Ming and Song, 2005) and develop into mature granular neurons (Ming and Song, 2005; Shapiro and Ribak, 2005; Zhao et al., 2006). The pool of NSCs is a potential resource for repairing the damaged hippocampus following TBI.

Bromodeoxyuridine (5-bromo-2-deoxyuridine; BrdU) labeling experiments have suggested that TBI promotes cell proliferation in the adult hippocampus (Braun et al., 2002; Chirumamilla et al., 2002; Dash et al., 2001; Kernie et al., 2001; Ramaswamy et al., 2005; Rice et al., 2003; Rola et al., 2006; Sun et al., 2005, 2007; Yoshimura et al., 2003). There are distinct classes of NSCs in the adult HDG, including quiescent neural progenitors (QNPs), which carry stem cell properties, and their progeny, amplifying neural progenitors (ANPs) (Bull and Bartlett, 2005; Encinas and Enikolopov, 2008; Encinas et al., 2006, 2008; Filippov et al., 2003; Mignone et al., 2004; Seaberg and van der Kooy, 2002; Seri and Garcia-Verdugo, 2001). We found that moderate TBI promotes proliferation of QNPs in the adult hippocampus (Gao et al., 2009).

Although TBI promotes NSCs proliferation, the effect of TBI on neurogenesis is still controversial. There are conflicting reports about neurogenesis in the HDG. According to some studies neurogenesis decreases after TBI (Braun et al., 2002; Rola et al., 2006), whereas others have reported that it remains unchanged (Chirumamilla et al., 2002; Rice et al., 2003), or that it increases (Sun et al., 2005, 2007). The experimental procedure of these studies was to inject animals daily with BrdU consecutively for 7 days (i.p. once per day). This procedure assessed the cumulative effect of TBI on cell proliferation. It did not address what are those proliferating cells, when cell proliferation gets started, how long the promoted proliferation lasts, how many of those proliferating cells survive, and what kind of cell they differentiate into. Furthermore, besides its action on NSCs, TBI is known to induce proliferation of reactive astrocytes (Floyd and Lyeth, 2007; Sandhir et al., 2008). Since there is a large number of reactive glial cells labeled by BrdU as well, it is possible to mistake gliogenesis for neurogenesis following TBI. To address these questions, here we traced the fate of those proliferating cells following TBI in the combination of BrdU

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labeling, multiple cell-type specific markers, transgenic mouse, and microscopy with 3-dimensional reconstruction.

Materials and methods

Animal care

Male C57 BL/6 mice (Jackson Laboratories) were group-housed and kept in a 12/12-hour light/dark cycle with free to access to food and water *ad libitum*. The nestin-EGFP transgenic mice (C57/BL6) were kindly provided by Dr. Enikolopov at Cold Spring Harbor Laboratories and described previously (Mignone et al., 2004). The animals were used in experiments at an age of 8 weeks. All procedures were performed under protocols approved by Indiana University's Animal Care and Use Committee.

Controlled cortical impact traumatic brain injury

Eight-week-old mice were subjected to moderate, controlled cortical impact injury (CCI) or sham surgery as previously described (Gao and Chen, 2008; Gao et al., 2008; Hall et al., 2004, 2005; Saatman et al., 2006; Sullivan et al., 1999a,b). Briefly, the mice were anesthetized and placed in a stereotaxic frame (Kopf Instruments, Tujunga, CA) prior to TBI. Using sterile procedures, the skin was retracted and a 4 mm craniotomy centered between the lambda and bregma sutures was performed. A point was identified midway between the lambda and bregma sutures and laterally midway between the central suture and the temporalis muscle. The skullcap was carefully removed without disruption of the underlying dura. Prior to injury induction, the tip of the impactor was angled and kept perpendicular to the exposed cortical surface. The mouse CCI model uses an electromagnetic impactor that allows one to alter the severity of the injury by controlling contact velocity and the level of cortical deformation independently. In the experiments for this study, the contact velocity was set at 3.0 m/s and deformation at 1.0 mm. These settings will result in an injury of moderate severity. Following injury induction, a 4 mm wide disk made of dental cement (Dentsply Trubyte, Johnson and Johnson, Arlington, TX) was placed over the craniotomy site, adhered to the skull using cyanoacrylate, and allowed to dry prior to suturing of the wound. During surgery and recovery, the core body temperature of the animals was maintained at 36–37 °C using a heating pad. Sham (noninjured) animals were subjected to craniotomy, but did not receive a CCI injury.

Pulse-labeling of the proliferating cells following TBI

The mice were subjected to moderate TBI or sham surgery as described, followed by administration of BrdU immediately after (time 0), and at 20 h, 44 h, 68 h, and 1 week following TBI (5 mice for each time point; BrdU: 100 mg/kg in saline, i.p.; Sigma, St. Louis, MO). The mice were perfused 4 h after BrdU injection to assess proliferation of NSCs and activated astrocytes.

Determining the fate of the proliferating neural stem/progenitor cells following TBI

Eight-week-old male C57/BL6 mice were subjected to moderate TBI or sham surgery as described. The sham and the injured mice (5 animals for each group) were given bromodeoxyuridine (BrdU) injections once per day for 7 days (50 mg/kg in saline, i.p.; Sigma, St. Louis, MO). Four weeks after the final BrdU injection the brains were fixed to evaluate the differentiation of BrdU-labeled cells in the hippocampus.

Tissue processing

The animals were deeply anesthetized and then perfused transcardially with cold saline, followed by a fixative containing 4% paraformaldehyde (PFA) in PBS. The brains were removed, post-fixed overnight in PFA, and cryoprotected for 48 h in 30% sucrose. Serial 30 μm thick coronal sections were cut using a cryostat (Leica CM 1950), and stored at −20 °C. The sections were then processed for immunohistochemical analysis.

Immunohistochemistry

Every sixth section (180 μm interval), covering the distance of the hippocampus, was processed for immunohistochemical analysis. Free-floating sections were washed twice in PBS, incubated in 2N HCl for 1 h at room temperature, and then soaked in 0.1 M borate buffer for 10 min (pH 8.4). After washing with PBS (3 times), the sections were incubated in blocking solution (0.1% Triton X-100, 1% bovine serum albumin, and 5% normal goat serum in PBS) for 1 h at room temperature, followed by overnight incubation with primary antibody at 4 °C. The sections were washed again with PBS (3 times), and incubated at room temperature for 2 h with the secondary antibody. After treatment (2 min) with DAPI (4′,6-diamidino-2-phenylindole), the sections were washed with PBS (3 times), and mounted using Fluorescent Mount G. Primary antibodies and their final concentrations were as follows: anti-BrdU (1:400, rat, Accurate Chemical and Scientific), anti-NeuN (1:1000, mouse, Millipore), anti-nestin (1:1000; rabbit; Covance, Berkeley, CA), anti-GFAP (1:100, rabbit, Sigma), anti-Iba-1 (1:200, goat, Abcam), anti-EGFP (1:1000, rabbit, Invitrogen), anti-S100 (1:200, rabbit, Sigma). Secondary antibodies from Jackson ImmunoResearch Laboratories, Inc. were applied in a dilution of 1:1000.

Cell counting

Immunohistochemistry was performed simultaneously on sections to detect the target cells. Series of every sixth section (30 μm thickness, 180 μm apart) through each hippocampus were processed. The cell density was determined through a blinded quantitative histological analysis. The profile count method was used. Every single BrdU-positive cell (even the partial of BrdU-positive nuclei at the border of section), or BrdU and specific cell marker (NeuN for mature neurons and GFAP for astrocytes) double-labeled cell in the different subregion of the dentate gyrus (including the molecular layer [ML], the granule cell layer [GCL], the subgranular zone [SGZ], and the hilus) in the multiplanes throughout the entire 30 μm section, was counted under a fluorescent microscope using the 40× objective through a whole series of sections.

The double-labeled cell was determined as follows. We used BrdU as an indicator. When the BrdU-positive cell showed up, we switched to the channel matching the cell specific marker. If the target cell also had been marked, we considered it as double-labeled cell. The total number of quantified cells was justified by correction (Coggeshall and Lekan, 1996). The contours of the dentate gyrus area and each subregion were created, and the volume was measured using Bioquant software (Nashville, TN). BrdU-positive or double-labeled cells were expressed as average number/mm^3 ($n = 5$ for each group).

Microscopy

The sections were analyzed using an inverted microscopy system (Zeiss Axiovert 200 M) combined with apotome and interfaced with a digital camera (Zeiss Axio Cam MRc5) controlled by a computer. Images were captured using apotome in software (AxioVision, v4.8) and assembled and labeled in Photoshop 7.0 (Adobe Systems).