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Traumatic brain injury reveals novel cell lineage relationships within the subventricular zone☆

Gretchen M. Thomsen[a,1], Janel E. Le Belle[b,1], Jessica A. Harnisch[a], Whitney S. Mc Donald[a], David A. Hovda[a,c], Michael V. Sofroniew[d], Harley I. Kornblum[b,e,f,g,*], Neil G. Harris[a,**]

[a] The UCLA Brain Injury Research Center, Department of Neurosurgery, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
[b] NPI-Semel Institute for Neuroscience & Human Behavior, Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
[c] Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
[d] Department of Neurobiology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
[e] Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
[f] Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
[g] Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA

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Abstract The acute response of the rodent subventricular zone (SVZ) to traumatic brain injury (TBI) involves a physical expansion through increased cell proliferation. However, the cellular underpinnings of these changes are not well understood. Our analyses have revealed that there are two distinct transit-amplifying cell populations that respond in opposite ways to injury. Mash1+ transit-amplifying cells are the primary SVZ cell type that is stimulated to divide following TBI. In contrast, the EGFR+ population, which has been considered to be a functionally equivalent progenitor population to Mash1+ cells in the uninjured brain, becomes significantly less proliferative after injury. Although normally quiescent GFAP+ stem cells are stimulated to divide in SVZ ablation models, we found that the GFAP+ stem cells do not divide more after TBI. We found, instead, that TBI results in

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* Correspondence to: H. Kornblum, Departments of Psychiatry and Biobehavioral Sciences, Pharmacology and Pediatrics, David Geffen School of Medicine at UCLA, Neuroscience Research Bldg., Suite 379, 635 Charles E. Young Drive South, Los Angeles, CA 90095-7332, USA. Fax: +1 310 206 5061.
** Correspondence to: N.G. Harris, Department of Neurosurgery, David Geffen School of Medicine at UCLA, Box 957039, Los Angeles, CA 90095-7039, USA. Fax: +1 801 672 1018.
E-mail addresses: hkornblum@mednet.ucla.edu (H.I. Kornblum), ngharris@mednet.ucla.edu (N.G. Harris).
[1] Co-first authors: Dr. Thomsen & Dr. Le Belle contributed equally to this manuscript.

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increased numbers of GFAP+/EGFR+ stem cells via non-proliferative means—potentially through the dedifferentiation of progenitor cells. EGFR+ progenitors from injured brains only were competent to revert to a stem cell state following brief exposure to growth factors. Thus, our results demonstrate previously unknown changes in lineage relationships that differ from conventional models and likely reflect an adaptive response of the SVZ to maintain endogenous brain repair after TBI.

Introduction

While increased proliferation and an expansion in the size of the SVZ are well-known phenomena after brain injury, the cellular underpinnings of this effect are not well understood. In addition, although injury-induced neurogenesis has been detected in the adult brain from the subventricular zone (SVZ) and hippocampus (Gould and Tanapat, 1997; Yagita et al., 2001; Parent et al., 2002; Thored et al., 2006) and in non-neurogenic regions (Tonchev et al., 2003; Yamamoto et al., 2001; Magavi et al., 2000) the regenerative capacity of the brain remains low (Arvidsson et al., 2002). Thus, therapeutic intervention aimed at certain cell populations or within specific time-frames post-injury are needed to enhance and support the endogenous neurogenic response.

Under uninjured conditions, stem cells in the SVZ are a relatively quiescent population of cells (Morshead et al., 1994; Doetsch et al., 1999a,1999b; Garcia et al., 2004; Imura et al., 2003), while transit-amplifying progenitors (Doetsch et al., 2002; Cesetti et al., 2009; Kim et al., 2009) and some neuroblasts (Brown et al., 2003) are populations of actively proliferating cells. According to the current models of SVZ lineage progression, the development from slowly-dividing GFAP+ stem cell to migrating neuroblast occurs following activation and co-expression of epidermal growth factor receptor (EGFR). These GFAP+/EGFR+ stem cells give rise to GFAP−/EGFR+ transit amplifying cells, which rapidly divide to generate DCX+ neuroblasts and continue to divide as they migrate toward the olfactory bulb where they become functional interneurons (Pastrana et al., 2009). Although studies utilizing the deletion of specific SVZ cell populations demonstrate this specific pattern of cellular hierarchy in the uninjured brain, it is unknown whether injury-induced SVZ cell proliferation involves changes to this normal lineage progression, and which specific cell phenotypes are most affected. Resolution of this post-injury biology is important for understanding the ability of SVZ-derived stem and progenitor cells to contribute to the brain's natural repair process.

To address this gap in knowledge we examined the cellular changes within the SVZ after traumatic brain injury (TBI) in a murine model. We identified a significant non-proliferative increase in neural stem cells and a divergent response to injury by different transit amplifying progenitor populations. Our data also suggest that injury-induced signaling through the EGF receptor may result in the dedifferentiation of a progenitor population back into a stem cell state. Thus, these alterations to cell lineage relationships in the SVZ are likely to be important regulators of the enhanced proliferation and neurogenesis known to be induced by brain injury. Therefore, EGFR-signaling in particular may be an important therapeutic target for optimizing the post-injury cellular response to promote functional recovery.

Materials and methods

Animals

C57Bl/6 mice purchased from Charles River Laboratory were housed under NIH guidelines and all experiments were conducted in accordance with the University of California, Los Angeles, (UCLA) Chancellor's Animal Research Committee and the Public Health Service Policy on Humane Care and Use of Laboratory Animals. Transgenic mice expressing the herpes simplex virus-thymidine kinase from the mouse glial fibrillary acid protein promoter (GFAP-TK mice) were supplied by the Sofroniew Lab at UCLA. The pattern and regulation of transgene-derived HSV-TK expression is similar to that of endogenous GFAP, to the extent that 100% of TK cells co-localize with GFAP in both uninjured mice (Garcia et al., 2004) or in stab wound-injured mice (Bush et al., 1998). Adult male mice at least 3 months of age were used in all experiments.

5-Chloro-2′-deoxyuridine (CldU) labeling

To label cells that were actively proliferating on the day of euthanasia (1, 3, or 7 days post-injury), 42.5 mg/kg 5-chloro-2′-deoxyuridine (CldU) was administered intraperitoneally every 2 h over the course of 8 h (4 injections total) and mice were euthanized 2 h after the last injection. To identify GFAP-TK+ cells that arise from actively dividing cells after injury, animals were injected with CldU immediately after injury and every 2 h thereafter for a total of 4 injections and animals were euthanized 3 days following injury.

5-Iodo-2′-deoxyuridine (IdU) labeling

For label-retaining experiments, intraperitoneal (IP) injections of IdU (Sigma I7125; 57.5 mg/kg) were administered to adult mice, once daily for three weeks to label all dividing cells, even the slowly dividing stem cells. Naïve animals were euthanized immediately or after a label washout period of 10 days in which no injections were given. Over this 10 day wash out period the IdU label intensity within proliferating cells will diminish by half with every division, so that fast dividing cells become dim or undetectable and quiescent cells remain brightly labeled (see Results). Animals in the injury group received a TBI 7 days after