Activating Endogenous Neural Stem Cells for Traumatic Brain Injury Treatment

Jeremy Anderson1, and Li Cai1
1Department of Biomedical Engineering, Rutgers The State University of New Jersey, Piscataway NJ

Traumatic brain injury (TBI) affects people of all ages and can lead to temporary or permanent loss of motor and cognitive function. Current therapeutics minimize secondary injury but do not promote functional recovery. Neurogenesis in the adult brain can be induced by traumatic brain injury (TBI), indicating the potential of activating the endogenous neural stem cells (NSCs) in repair and regeneration. However, the mechanism of injury-induced neurogenesis remains to be determined. Further understanding the scope of NSC activation and genes driving NSC activation will improve understanding of genes driving neurogenesis after TBI and provide new therapeutic targets for patients suffering from TBI.

I. Introduction

TBI is defined as a mild to severe shock to the head that disrupts normal brain function. Immediately after TBI, cells die from the impact (primary injury). The primary injury is followed by bleeding and inflammation (secondary injury), which also causes increased cell death. TBI, which can result from sport injuries, vehicular accidents, violence, and falls, can lead to temporary or permanent loss of memory and motor function. TBI was responsible for 2.2 million emergency department visits and 50,000 deaths in 2014 (CDC, 2015).

Unfortunately, there is a gap of knowledge surrounding TBI, and there is currently no effective therapy for TBI. Most therapies addressing TBI are involved with minimizing the secondary injury and inflammation, which help reduce additional damage and cell death. Although such therapeutics improve the outcome of the TBI, they do not address the primary injury and cell death resulting from TBI, often leaving TBI survivors with loss of memory and motor function. Research has shown that TBI induces endogenous neural stem cell (NSC) activation. Although the primary injury cannot be reversed, research has moved towards promoting tissue regeneration after TBI through stem cell therapies and endogenous NSC activation. Stem cell therapies are currently in clinical trials but not approved because of the challenges and risks associated with stem cell therapies. Brain injury activates NSCs, with adult neurogenesis occurring in the subgranular zone (SGZ) of dentate gyrus (DG) in the hippocampus and the subventricular zone (SVZ) of the neocortex. Research has found increased neurogenic response in SVZ of DG post-TBI (1). However, the injury-activated cells are often insufficient to recover the cells lost from injury. Manipulating endogenous NSC activity after TBI to promote neurogenesis is an alternative method for promoting healing and improving functional recovery, and new neurons have been shown to integrate into neuronal circuitry and be involved in learning, memory, and motor function (1).

Our research aims to better characterize TBI-induced cell and transcriptome changes, specifically TBI-induced NSC states and their associated transcriptomes to better understand how NSCs respond to TBI, and what gene changes are driving this NSC response and activation to TBI. This research has translatability potential because the neurogenic response to TBI in rodents and humans expressed similar NSC protein markers (2). A better understanding of NSC response to TBI-induced injury will allow for new essential genes to be identified, new therapeutic or disease markers to be characterized, and new therapeutic targets to be identified, opening up the potential for the development of next generation therapeutics to improve outcomes for patients who suffer from TBI.

II. Main Results

To determine the mechanism of injury-induced neurogenesis, we employed a closed head injury (CHI) model in Notch1CR2-GFP transgenic mice (3, 4). We characterized NSC activation and brain injury markers after TBI through immunohistochemistry and qPCR. The CHI model has been chosen because it is commonly used and representative of 85-89% of clinical cases of TBI (5).

Notch1CR2-GFP transgenic mice have been used to validate our model and characterize the NSC activation after injury. The Notch gene is important for studying stem cell activity because Notch regulates the production of neural progenitor cells and neuroblasts in embryos and adult animals. In these transgenic mice, activity of the CR2 region in the Notch gene directs GFP expression exclusively in NSCs (4). A noticeable increase in GFP expression in the dentate gyrus of the hippocampus, the ependymal cells lining the lateral ventricle, and the olfactory bulb was observed in the injured animals as compared to the control and sham animals at 1, 2, 3, 5, and 7 day(s) post injury (dpi). Injury-induced GFP+ cells were co-localized with NSC marker Nestin, neuronal progenitor marker DCX, and neuronal marker NeuN, indicating TBI activates NSCs and induces neurogenesis.

Figure 1. CHI induces NSC activation in the hippocampus. Photomicrographs of GFP+ cells in the dentate gyrus of the hippocampus are depicted. Increased number of GFP+ cells in the injured brain is indicative of neural stem cell activation post-injury.
Quantification of gene expression in brain tissue by quantitative real-time PCR (qPCR) has also demonstrated an increase of brain injury markers (e.g., Tau, ApoE) and inflammatory interleukins (e.g., Interleukin-1β) in the injured tissue compared to sham and control tissues after injury. The reference gene (housekeeping gene) GAPDH was used for normalization. qPCR results were analyzed by the Livak method (ΔΔCT method) to calculate fold difference in expression in injured tissues.

III. Materials and Methods

NSC activation post-TBI using a closed head injury (CHI) model (4 cm drop height, 327 g drop weight) with a transgenic Notch1CR2-GFP mouse was examined to characterize NSC proliferation, migration, and differentiation. Transgenic mice 8-12 weeks of age were injured using the CHI model. The mice were analyzed at 1, 2, 3, 5, and 7 day(s) post injury. Mice were screened for GFP expression in the hippocampus, lateral ventricle, and olfactory bulb – known regions of higher NSC presence. Brain tissue was preserved and stained for GFP, NeuN, DCX, and Nestin to determine cell identity, and GFP+ cells were counted to compare differences in GFP expression between injured and uninjured animals. For qPCR analysis, brain tissue was removed from the mice, cells were dissociated, RNA was isolated, cDNA was synthesized, and qPCR was performed.

IV. Conclusions

Increased number of GFP+ cells and co-labeling with NSC marker Nestin in the brain post-TBI indicates NSC activation. There is also increased Interleukin, ApoE, and Tau RNA expression in injured brains. These results establish that our transgenic animal CHI model is a useful tool for further cellular and molecular studies of TBI. It will be helpful to elucidate the mechanisms of NSC activation after injury.

Acknowledgements

J.A. thanks Li Cai and the Cai Lab for support throughout this project. The work was supported in part by grants from the New Jersey Commission on Spinal Cord Research, Busch Biomedical Research, and a National Institutes of Health funded Biotechnology Training Program Fellowship (T32GM008339).

All research was performed under compliance with Ethical Standards and the authors declare that there is no conflict of interest.

References