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Multimodal imaging of Hair Follicle Bulge-Derived Stem Cells in Brain Injury Mice

Introduction

Traumatic brain injury (TBI) is a devastating event for which current therapies are limited. During the recent years, stem cell (SC) therapy has attracted a huge interest as a new therapeutic method for TBI treatment. Implantation of autologous SCs can lead to recovery of function via different mechanism, such as cell replacement via differentiation, stimulation of angiogenesis and support to the microenvironment.
Adult hair follicle bulge-derived stem cells (HFBSCs) possess neuronal differentiation capacity, are easy to harvest and relatively immune-privileged, which makes them potential candidates for TBI treatment.
In this preliminary study, we applied *in vivo* multimodal, optical and MR, imaging techniques to investigate the behavior of mouse HFBSCs in a TBI model.

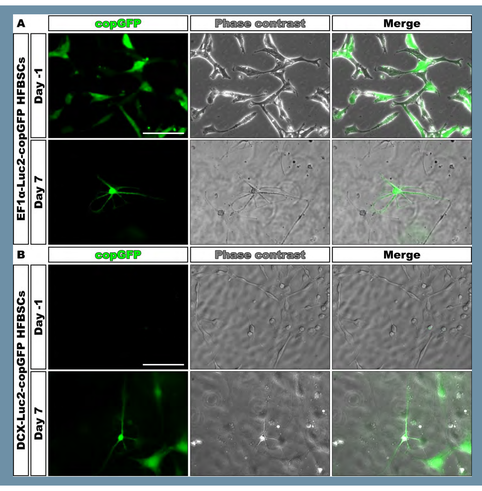
Experimental Setup

HFBSCs were isolated and transduced with a lentiviral reporter gene construct containing Luc2-T2A-copGFP regulated by either a EF1α- or DCX-promoter to constitutively express equimolar amounts of Luc2 luciferase and copGFP. Traumatic brain injury (TBI) was induced in nude mice by application of a liquid nitrogen pre-cooled copper conical cylinder to the head of each mouse approximately 3 mm left of the bregma for 40 seconds. Animals were allowed to recover for 2 days before approximately $2x10^5$ HFBSCs were injected into the cerebral cortex of nude mice. Cells were preloaded with a ferumoxytol-heparin-protamine complex and implanted next to the lesion area (in the cortical region) 2 days after induction of injury. Cells were visualized using bioluminescence imaging (BLI) weekly. MRI was performed the day after implantation and again after 48 days, whereupon brains were fixed for immunohistochemistry (IHC).

1. *In vitro* differentiation of transduced HFBSCs

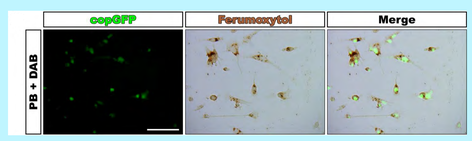
(A) Before differentiation (Day -1) EF1α-Luc2-T2A-copGFP-transduced cells exhibit mostly bright fluorescence (copGFP) and normal morphologies (Phase contrast). The merged image demonstrates that all cells were transduced with the reporter gene construct. Within 7 days, HFBSCs adapted neuronal morphologies. Scale bar is 100 µm.

(B) HFBSCs transduced with the DCX-Luc2-T2A-copGFP construct do not express copGFP (or Luc2) prior to differentiation (Day -1) as indicated by the absence of fluorescence. However, 7 days after start of the differentiation, cells express copGFP under regulation of the DCX promoter. This reveals parallel expression of the microtubule-associated protein doublecortin, which is primarily expressed by neuronal precursor cells and immature neurons. Scale bar is 100 µm.



2. Loading of HFBSCs with Ferumoxytol

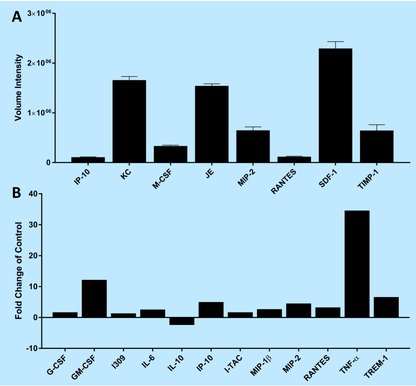
To investigate whether HFBSCs take up the FDA-approved ferumoxytol in a ferumoxytol-heparin-protamine complex, we loaded transduced cells with these iron oxide nanoparticles.
Perl's Prussian Blue staining + DAB intensification reveals iron oxide (Ferumoxytol) within the cells. CopGFP-fluorescence persists through staining process as visible in merged image. Scale bar is 100 µm.



3. Cytokine expression of HFBSCs

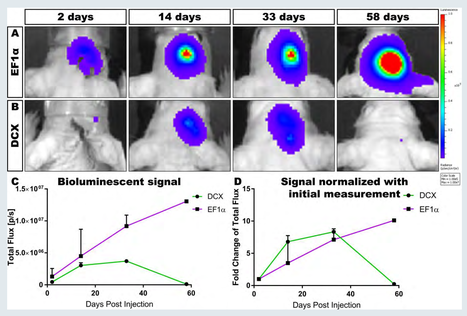
(A) Cultured, unchallenged HFBSCs secrete a high concentration of TIMP-1, which was reported to promote oligodendrogenesis and myelination in general, but also SDF-1 that is strongly chemotactic for lymphocytes. Similar to SDF-1, the other factors (IP-10/CXCL10, KC/CXCL1, M-CSF/CSF-1, JE/MCP-1, MIP-2/CXCL2, RANTES/CCL5) are all involved in recruitment of immune cells, such as T cells, macrophages, neutrophils and dendritic cells.

(B) Change in secretion of factors in response to TNFα-challenge. In response to TNFα various factors become expressed differently, like GM-SCF and IP-10 (5-10-fold increase) that are responsible for the recruitment of immune cells. HFBSCs also produce more IL-6, which is immunomodulatory.



4. Observation of Luc2 activity in TBI mice *in vivo*

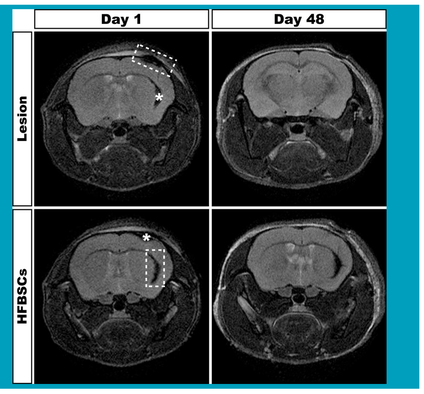
(A) Representative overlays of EF1α-Luc2-T2A-copGFP-transduced HFBSCs starting 2 days after transplantation until day 58. The bioluminescence signal increased relatively stable over the course of time. **(B)** Representative overlays of HFBSCs transduced with DCX-Luc2-T2A-copGFP over the same period of time. 2 days after transplantation, no bioluminescent signal could be observed. However, the signal increased after 14 days and 33 days, but vanished after 58 days, which is in line with the expression pattern of DCX during neuronal differentiation. **(C)** Bioluminescent signal was measured 2, 14, 33 and 58 days after transplantation. **(D)** Same bioluminescence data was normalized with the initial signal measured 2 days after transplantation.



5. MRI of ferumoxytol-loaded HFBSCs in TBI mice *in vivo*

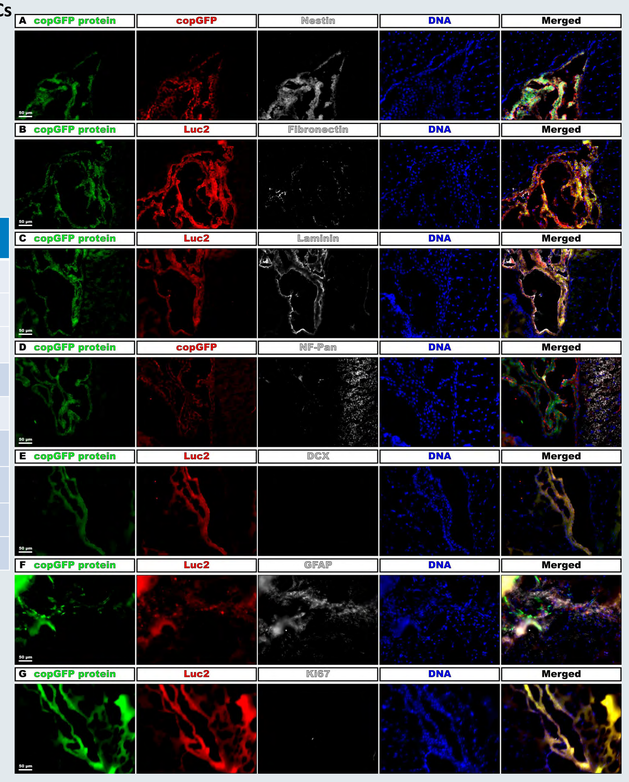
The upper panel shows representative scans of the region containing the TBI lesion 1 day and 48 days after transplantation. The scan at day 1 reveals a hypointense area containing clotted blood (dotted box), which vanished over the course of time. Asterisk represents injected cells.

The lower pane shows representative scans of the injected ferumoxytol-loaded, transduced HFBSCs over the same course of time (asterisk). The dotted box at day 1 after injection reveals the location of iron-oxide-containing cells. Loaded HFBSCs can be imaged for at least 48 days after injection.



6. Immunohistochemical staining of copGFP-expressing HFBSCs

	Antibody	HFBSCs	Transplanted HFBSCs	Specification
HFBSCs	copGFP	positive	positive	Transduced HFBSCs expressing copGFP
	Luc2	positive	positive	Transduced HFBSCs expressing Luc2
Neural Crest	Nestin	positive	positive	Neural crest cells and neuronal progenitors
Neuron	DCX	negative	weakly positive	Early neuronal development
	NF-Pan	weakly positive	positive	Neurons
Glial Cell	GFAP	negative	negative	Glial cells in the peripheral and central nervous system
ECM	Fibro-nectin	negative	positive	Cell adhesion, growth, migration, differentiation, neuron protection
	Laminin	positive	positive	Cell attachment, stimulates neuronal differentiation, promotion of tissue survival
Other	Ki67	positive	negative	Proliferating cells



Conclusion

Constant BLI signals from the area of implantation into the mouse brain indicated good survival of implanted HFBSCs during the study. It was possible to visualize cells in the injured brain by means of MRI, 1 day and 48 days after transplantation. Most of the cells remained at the implantation site, while some cells migrated towards the area of TBI. IHC revealed staining of implanted cells for pan-neurofilament (NF) but not for glial fibrillary acidic protein (GFAP). The majority of cells stained for nestin. Cells were producing laminin and fibronectin but no extracellular matrix (ECM) masses were detected.
These results suggest that HFBSCs are able to survive and differentiate towards a neuronal cell lineage after brain implantation and support their potential use for cell-based therapy of traumatic brain injury. Further studies are needed to elucidate their ability to enhance endogenous repair processes.