

Brain Ischemia and Regeneration



Raphael Guzman
Department of Biomedicine
Division of Neurosurgery
University Hospital Basel
University Children's
Hospital Basel

Group Members

Dr. Catherine Br  g  re
(Postdoc)
Pia Bustos Meneses
(Technician)
Dr. Laurie Chicha*
(Postdoc)
Tanja Dittmar
(Technician)
Dr. med. Urs Fisch
(MD-PhD Student)
Alain Huber
(Undergraduate Student)
Kirill Karlin*
(Undergraduate Student)
Wolfgang-Sebastian Lieb*
(Undergraduate Student)
Dr. Srinivas Madduri
(External Collaborator)
Stephan Moser*
(Undergraduate Student)
Dr. med. Viktoria Pfeifle
(Postdoc)
Sahar Rosenblum*
(MD Student)
Dr. Michel R  thlisberger
(Postdoc)
Fabio Roveri*
(PhD Student)

Dr. Bernd Schwendele
(Postdoc)
Tenille Smith*
(MD Student)
*left during report period

Neonatal Hypoxia-Ischemia . Stroke . White Matter Regeneration . Neural Stem cells
Microglia . CSF Biomarkers

Therapeutic relevance of neural stem cells for white matter regeneration in neonatal hypoxic-ischemic brain injury

Neonatal hypoxic-ischemic (HI) brain injury is the result of an impaired delivery of oxygen and/or blood to the infant's brain. One of its typical hallmark is white matter damage, which can severely affect the development of the brain, leading to devastating sensory-motor, cognitive and learning deficits in the growing child. Currently no available therapy targets the long-term consequences of early brain injury, making regenerative medicine a promising therapeutic option. Several reports suggest that transplanted neural stem cells (NSCs) promote CNS tissue repair not only through cell replacement, but also by providing trophic and immunomodulatory support for endogenous repair mechanisms, including neurogenesis and oligodendrogenesis. A major focus of our research aims at investigating the cellular and molecular mechanisms underlying the neuroprotective role of transplanted NSCs in the context of developmental brain injuries.

Impact of NSCs on oligodendrocyte progenitor cells (OPCs)

We have promising preliminary data showing that endovascular injection of human embryonic stem cell (ESC)-derived NSCs improve both sensory-motor and cognitive functions in a rodent model of neonatal HI. We observed that NSCs treatment specifically stimulates white matter repair mechanisms such as OPCs proliferation and maturation with significant increase in myelin basic protein expression. Nevertheless, the specific molecular mechanism by which NSCs exert this beneficial effect on OPCs are currently unknown. To address this issue, we are performing *in vitro* experiments testing the impact of the conditioned medium from human NSCs onto OPCs cultures (Fig. 1) as well as direct cellular interaction between NSCs and OPCs. Our objective is to identify the specific trophic factors released by NSCs that can influence the oligodendroglial lineage.

Interaction between microglia and NSCs

NSC-mediated regeneration may also occur through immunomodulation of microglia, the immune cells of the brain. Indeed, our research points to a direct interaction between NSCs and microglia through NSC-secreted factors *in vitro* and *in vivo*, in the healthy animals, as published in Mosher *et al.*, 2012. To further understand this interaction, we are investigating the impact of a neonatal HI brain injury on the phenotype of microglia in the subventricular zone (SVZ), one of the neurogenic niches (Fig. 2). The hypothesis is that SVZ microglia adopt a pro-neurogenic phenotype, which might contribute to CNS regeneration following HI. Our histological data show that the phenotype of microglia undergoes dramatic changes postnatally, and that the HI insult impacts durably these physiological changes. Transcriptomic analyses indicate that HI-exposed SVZ microglia adopt a complex phenotype resembling that observed in neurodegenerative diseases, an intriguing finding that merits further investigation. The implications for SVZ neurogenesis *in vivo* remain to be explored.

Developing matrices to optimize NSCs survival into the host tissue

NSCs are typically delivered in a medium solution such as saline. However, once injected, transplanted NSCs show limited survival into the host tissue, thus significantly shortening their therapeutic time-window. To overcome this issue, NSCs can be incorporated in biocompatible matrices. The advantage of such matrices is that they provide physical support for the NSCs, and they can be engineered to carry trophic factors. This research aims at developing matrices that ameliorate NSCs survival, and eventually potentiate central and peripheral neural tissue regeneration. This work is done in collaboration with Prof. D. Kalbermatten and Dr. S. Madduri.

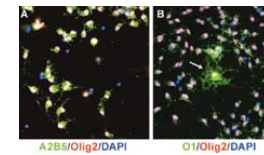


Fig. 1: *In vitro* maturation of oligodendrocyte progenitor cells (OPCs). Representative 20 X photomicrographs of enriched cultures of OPCs isolated from 2-days old rat neonates. (A) shows OPCs stained with A2B5 (green), a marker of immature OPCs, and Olig2 (red), a nuclear marker of the whole oligodendroglial lineage. (B) shows maturing OPCs labeled with O1 (green), and Olig2 (red). Nuclei are counterstained with DAPI (blue). During maturation, the morphology of the OPCs evolves from a bipolar to a more ramified, complex shape (depicted by the white arrow on B). (Scale = 20 μ m)

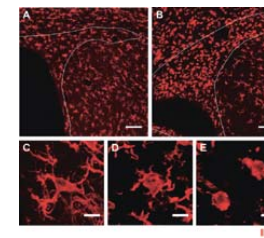


Fig. 2: Iba1-positive microglia are more numerous, and become activated in the SVZ three days after neonatal HI in rat. Representative 20X photomicrographs showing Iba1 immunostaining (red) in the SVZ (outlined in white) from sham neonates (A) and in the ipsilateral SVZ from HI neonates (B). Microglial activation is reflected in changes in cellular morphology, from a resting-ramified state (C) to an intermediate (D) and a highly active-amoeboid state (E). (Scale = 100 μ m for A and B; scale = 10 μ m for C, D and E)

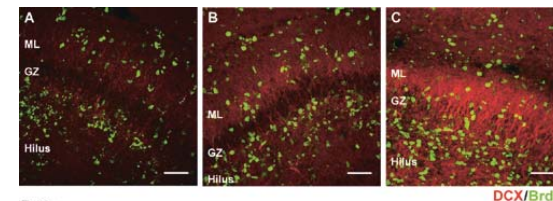


Fig. 3: Neurogenesis and proliferation are stimulated in the hippocampal dentate gyrus (DG) three days after neonatal HI in rat. Representative 40X confocal photomicrographs showing double immunostaining for DCX (red) and BrdU, a proliferation marker (green) in the DG of sham neonates (A), and in the contralateral (B) and ipsilateral (C) DG from HI neonates. (D) Graph showing that the percentage of double positive cells is significantly higher in the injured-ipsilateral DG than in the contralateral and sham DG. (Scale = 50 μ m; * denotes $p < 0.05$, and *** $p < 0.001$)

Our research is funded by the Swiss National Found (SNF) grant 146632 and SNF grant 31003A_163305 in collaboration with the Fetal and Neonatal Stress Research Group of Prof. S. Wellmann at UKBB. The tissue engineering project is supported by a research grant from the Department of Surgery in collaboration with Prof. D. Kalbermatten and Dr. S. Madduri. We have active international collaboration with Stanford University (United States), Newcastle University (England) and the University of Saskatchewan (Canada).

Connection to Clinical Practice

CSF markers of endogenous regenerative processes in the developing brain

Our group teamed up with the CNS Discovery Department of Hoffmann-La Roche to evaluate potential cerebrospinal fluid (CSF) biomarkers with a predictive value for neurodevelopmental impairments, as there is currently an unmet clinical need for such markers. One of our candidate biomarkers was doublecortin (DCX), a CNS protein considered as a marker of neurogenesis in the brain (Fig. 3). Through the use of a novel immunoassay (developed by Roche) that allows quantification of doublecortin, we examined the relevance of this protein in the CSF, using the rat model of neonatal HI. Our investigations revealed that doublecortin in the CSF was both a reflector of stroke severity and of HI-induced neurogenesis. We are currently exploring the translational value of these findings by analyzing doublecortin and other CNS biomarkers in the CSF of pediatric patients.

Selected Publications

- Rosenblum S, Smith TN, Wang N, Chua JY, Westbroek E, Wang K, and Guzman R. (2015) BDNF pretreatment of human embryonic-derived neural stem cells improves cell survival and functional recovery after transplantation in hypoxic-ischemic stroke. *Cell Transplant* 24, 2449–2461.
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- Mosher KI, Andres RH, Fukuhara T, Blieri G, Hasegawa-Moriyama M, He Y, Guzman R and Wyss-Coray T. (2012) Neural progenitor cells regulate microglia functions and activity. *Nat Neurosci*.