In vivo and Ex vivo MRI in Differentiating Autologous from Primary Stem cell Migration in the Normal Mouse Brain

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Introduction: Delivery of therapeutic molecules by stem cells into the brain opens an opportunity for the treatment of several neuronal diseases. It has been shown that intraventricular neonatal transplantation of an immortalized murine neuronal stem cell line (C17.2) results in extensive cell engraftment/migration and correction of storage lesions in a murine model of the lysosomal storage disease Mucopolysaccharidosis VII (Snyder et al. 1995). However, little is known about the pattern of migration of primary neural stem/progenitor cells (NSCs) in the mouse brain. In this study, we implemented in vivo and ex vivo MRI for non-invasive tracking of the C17.2 cell line, as well as primary murine NSCs. The donor cells were labeled with super paramagnetic iron oxide (SPIO) particles. MR images were correlated with Prussian blue staining for iron detection.

Methods: *Cell culture*: C17.2 cells were held on uncoated flasks and maintained in DMEM with 10% FBS and 5% horse serum. Murine primary NSCs, isolated from the subventricular zone of three postnatal day 3 mice pups were cultured in poly-D-lysine coated T25 flasks and maintained in DMEM: F12 supplemented with N2 and 20 ng/mL EGF, 20 ng/mL bFGF, and 5-µg/mL heparin. *Labeling of cells with iron oxide particles:* Cells were labeled with SPIO particles (25 and 250µg Fe/ml, Feridex, Berlex labs, NJ) as reported earlier (Frank, et al. 2002). To determine iron concentration per cell, separate batches of C17.2 cells were weighed and digested in nitric acid. Iron concentration was measured by inductively coupled plasma mass spectroscopy. *Intra-cranial implantation:* Neonatal C3H/SCID mice were cryo-anaesthetized and injected on the day of birth. Twelve mice were injected with C17.2 and 6 mice were injected with primary cells. Two µl of the labeled cell suspension ($4.9x10^4$ cells/µL) was injected into each lateral ventricle. *Imaging:* 3D gradient-echo images were acquired on a 4.7 T magnet using a 2.5 cm birdcage coil after 6-7 weeks of implantation. In vivo imaging parameters: TR/TE=100/4.5 ms, matrix=256x128x128, FOV=$2cm^3$, slab thk~15 mm, nt=4, total acquisition time~120 min, spatial resolution ~ 156µm. At the end of in vivo experiments, mice were sacrificed and perfused with 4% paraformaldehyde. Brains were removed and 3D gradient-echo imaging was performed on a 9.4T magnet. Ex vivo imaging parameters: TR/TE =100/10 ms, matrix=256x128x128, slab thk~20mm, FOV~2x1x1 cm, nt=28, total acquisition time~13 h, spatial resolution ~ 78 µm. *Histology:* 20 µm-thick sections of the brain were stained with Prussian blue for detection of iron particles.

Results: In order to optimize the in vivo detection of SPIO labeled cells with minimum iron concentration that was non-toxic to the cells, cells were labeled at two concentrations (25, 250 µg Fe/ml). The effective internalization of SPIO particles is shown in Figure 1 for the C17.2 cell line. At the lower concentration, it was not possible to detect these cells in vivo, however, the higher concentration (250 µg Fe/ml) allowed for detection of both C17.2 and primary NSCs (Figure 2). Figure 2 shows a similar pattern of hypo-intense areas for both C17.2 and primary murine NSCs. However, hypointense areas were detected throughout the rostro-caudal axis of the C17.2 injected brains, whereas MRI revealed hypointense areas primarily in the middle of the brain surrounding the hippocampus and within the lateral ventricles in mice injected with primary murine NSCs.

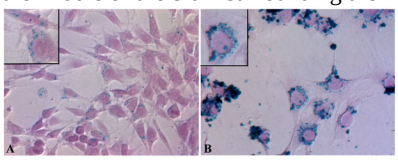

Fig: 1 Prussian blue staining of C17.2 cells at two different concentration of Feridex in incubation medium (magnification 10x). (A) 25µg of Feridex/ml; iron concentration 9.5 pg/cell. (B) 250µg Feridex/ml; iron concentration 26.3 pg/cell. Insert in each image show enlarged sections of a single cell

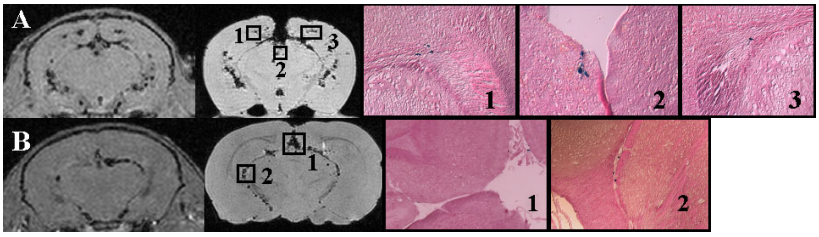

Fig: 2 *In vivo, ex vivo* and corresponding Prussian blue staining images of mouse brain after neonatal implantation of SPIO labeled (A) C17.2 cells and (B) mouse primary cells. The hypointense regions on in vivo and ex vivo images correlated well with the presence of iron oxide particles as shown by Prussian blue staining (boxes indicate the regions shown by Prussian blue staining (magnification 10x))

Discussion and Conclusion: Screening of SPIO labeled stem cells by in vivo MRI opens the possibility to study the migration capability of cells non-invasively. In vivo and ex vivo magnetic resonance images distinguished different engraftment patterns of C17.2 cells and mouse primary stem cells after 7 weeks of neonatal implantation in the normal mouse brain. Based on our previous study, low concentration (25 µg/ml) of iron in the labeling solution leads to the lack of in vivo detection of labeled cells in mouse brain (Magnitsky et al. 2004). Since, in vitro studies of cell viability demonstrated that a high iron concentration (250 µg/ml) in the labeling solution was not toxic and the cells divided in a normal fashion, we decided to use 250 µg/ml iron concentration for this study. Evaluation of MR images and histological sections showed widespread migration throughout the rostro-caudal axis of the entire brain when C17.2 cells were implanted. In contrast, murine primary NSC engraftment was more limited and restricted primarily to the center of the brain while minimal migration from the lateral ventricles into the septum, fimbria, hippocampus, and thalamus was detected. The MR images mirrored the histological results. The detection of different migration patterns indicates differences in engraftment between immortalized C17.2 cells and primary NSCs, and may facilitate stem cell based therapies of neuronal diseases.

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