

## Difference in Migration Pattern of Mouse and Canine Stem Cells Detected by in Vivo MRI

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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 global lesions in a murine model of the lysosomal storage disease Mucopolysaccharidosis VII [1]. However, the C17.2 cell line was immortalized by the introduction of a v-myc oncogene and therefore can not be used in clinical studies. Little is known about engraftment potency of primary mouse cells or stem cells obtained from different species. In this study, we used in vivo MRI for non-invasive tracking of the murine neuronal stem cells and canine progenitor cells after neonatal implantation into normal mouse brain. 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 maintained on uncoated T75 flasks, canine neural progenitor and mouse primary cells were maintained on poly-L-lysine coated T25 flasks. Murine primary neuronal stem cells (NSC's), isolated from the subventricular zone of three postnatal day 3 mice pups were maintained in DMEM: F12 (CellGro) medium and supplemented with 20 ng/mL EGF, 20 ng/mL bFGF, and 5- $\mu$ g/mL heparin. Canine neuronal progenitor cells (NPC's), isolated from subventricular zone of a neonatal dog were maintained in essentially the same medium as the murine cells. *Labeling of cells with iron oxide particles:* Cells were labeled with SPIO particles (250 $\mu$ g Fe/ml, Feridex, Berlex labs, NJ) as reported earlier [2]. *Intra-cranial implantation:* Neonatal C3H/SCID (n=10) mice were cryo-anaesthetized and injected on the day of birth. Two  $\mu$ l of the labeled C17.2, murine or canine cell suspension ( $4.9 \times 10^4$  cells/ $\mu$ L) was injected into each lateral ventricle and the animals were imaged 7 weeks later. *Imaging:* 3D gradient-echo images were acquired on a 4.7 T magnet using a 2.5 cm birdcage coil. In vivo imaging parameters: TR/TE=100/4.5 ms, matrix=128x128x128, FOV=2cm<sup>3</sup>, slab thk~15 mm, nt=4, total acquisition time~120 min, spatial resolution ~ 156 $\mu$ m. 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  $\mu$ m. *Histology:* 20  $\mu$ m-thick sections of the brain were stained with Prussian blue for detection of iron particles.

**Results:** Extensive migration of C17.2 cells was detected, while only limited migration of murine NCS and canine NPC cells was observed in vivo (Figure 1). The in vivo images were not able to detect subtle difference in migration pattern of mouse NSC's and canine NPC's (Figure 1 B, C). However, difference in engraftment potency of these cells was detected in ex vivo experiments (Figure 2).

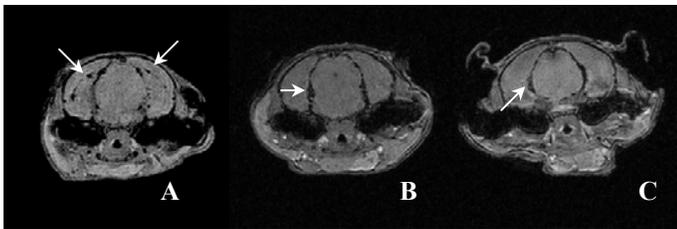


Figure 1 *In vivo* MRI images of mouse brain after neonatal implantation of SPIO labeled C17.2 cells (A), mouse progenitor NSC's (B) and canine NPC's (C) cells. In vivo imaging parameters: TR/TE = 100/4.5 ms, matrix = 128x128x128, slab thk = 16 mm FOV = 2.0 cm<sup>3</sup>, nt = 4, acquisition time ~ 120 min, spatial resolution ~ 156  $\mu$ m. Arrows indicate the hypointense regions due to presence of labeled cells.

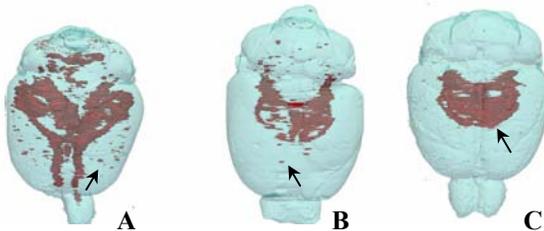


Figure 2 3D reconstruction of *ex vivo* MRI images of mouse brain after neonatal implantation of SPIO labeled C17.2 (A), mouse primary NSC's (B) and canine NPC's (C). Red areas represent labeled cells while light-blue is brain masks. Extensive migration of C17.2 cells is seen compared with more limited migration of murine NSC (arrow) and least migration of canine NPC's (arrow).

**Discussion and Conclusion:** Screening of SPIO labeled stem cells by in vivo MRI opens the possibility to study cells migration non-invasively. In vivo and ex vivo magnetic resonance images distinguished different engraftment potency of murine, canine and C17.2 stem cell after 7 weeks of neonatal implantation in the normal mouse brain. 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 [2]. 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. Canine progenitor cells implanted into normal mouse brain also exhibit limited migration and were found primarily in ventricular zone of the brain. The MR images mirrored the histological results. The detection of different migration patterns indicates differences in engraftment between immortalized C17.2 cells line and primary mouse or dog NSCs. This study confirms the recent finding that C17.2 cells has unique properties which can not be extrapolated to others NSC's [3].

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### Reference:

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