In vivo MRI of endogenous neural stem cell migration

E. M. Shapiro[1], O. Gonzalez[2], A. Alvarez-Buylla[2], A. P. Koretsky[1]

[1]Laboratory of Functional and Molecular Imaging, National Institutes of Health, Bethesda, MD, United States, [2]Neurological Surgery, University of California, San Francisco, San Francisco, CA, United States

Introduction

Cellular MRI is principally performed by labeling cells in culture with an intracellular contrast agent, transplanting into a host, and tracking the migration/homing with MRI. Potential problems associated with this, particularly with stem cells, is that cell culture cannot accurately reproduce the chemical and physical niche microenvironment stem cells reside in, and cell culture may actually alter the differentiation of stem cells from their natural fate. To avoid this, we have designed a protocol for labeling neural stem cells, in situ, using micron sized superparamagnetic iron oxide particles (MPIOs). This procedure takes advantage of the fact that only single MPIOs are required for robust visualization, thus allowing for inefficient labeling (1).

The subventricular zone (SVZ) is a neurogenic center in the rodent brain (2). Here, astrocyte-like neural stem cells lining the lateral cerebral ventricles produce neural progenitors which migrate within the rostral migratory stream (RMS) exclusively to the olfactory bulb (OB) (2). To label the neural stem cells, 1.63 micron MPIOs were injected directly into the lateral ventricle. Once particles are endocytosed by the neural stem cells, through asymmetric cell division, the particles can be transferred to the daughter neural progenitors and carried along during their migration. In vivo, high resolution, T_2^* weighted gradient-echo MRI was used to monitor this migration. Dark, punctuated contrast was present throughout the RMS, with the contrast fanning out in the granule cell layers of the OB. Histology and immunohistochemistry (IHC) were then performed to establish what cell type the particles were in along the entire migratory pathway. It was determined that particles labeled ependymal cells, microglia and neural stem cells at the ventricle, that neural progenitors were labeled along the RMS, and that new neurons were labeled in the OB. Cells were observed containing as few as single particles, and as many as 15 particles.

Materials and Methods

Neural stem cells were labeled by direct stereotactic injection of 5-50 µl of 1.63 micron MPIOs (10^{7-8} particles) into the lateral cerebral ventricle of six week old rats (n=24). Five additional rats received particles pre-soaked for 24 hours in EGF, a stem cell signaling chemical which causes neural stem cells to project cilia into the ventricular space (3). These MPIO's are polystyrene/divinylbenzene polymer coated iron oxide cores with a green fluorescent chemical impregnated into the shell (Bangs Laboratories, Fishers , IN). The measured relaxivity of these MPIO's at 4.7T was 356 $mM^{-1}s^{-1}$, while MION was 240 $mM^{-1}s^{-1}$ (4). 3D gradient echo images of live, anesthetized rats were acquired at time points over the course of six weeks with the following parameters: TR = 70 ms, TE = 8 ms, 100 micron isotropic across 2.56 cm^3. Imaging was done at 11.7 Tesla using a volume transmit and 3.5 cm receive only surface coil. Rats were then perfused, fixed with 1 mM Gd-DTPA doped 4% formaldehyde, and the brains with intact olfactory bulbs were removed. Brains were then placed in saline and imaged overnight at 50 microns isotropic resolution. Following imaging, brains were sectioned for immunohistochemistry (IHC) to determine the types of cells the particles were in. Antibodies used for IHC included anti-S100B (ependymal cells), anti-Iba1 (microglia), anti-GFAP (astrocytes and neural stem cells), anti-PSA-NCAM (neural precursors), anti-doublecortin (new neurons), anti-Dlx2 (intermediate neural stem/precursor cells), anti-NeuN (mature neurons) and anti-Tuj1 (mature neurons). Red fluorescent secondary antibodies were used to visualize cell types. Some sections were iron stained with Prussian Blue. To aid in determining the migration path for histology, some rats were injected in the lateral ventricle with BrdU, with appropriate histology one week later to determine the RMS.

Results

Figure 1 shows MR images of the RMS and OB in the same rat brain A) *in vivo* immediately following an EGF-soaked 50 µl MPIO injection, B) *in vivo* five weeks after MPIO injection, and C) *in vitro* five weeks after MPIO injection. Immediately after injection, the ventricles are completely dark due to free MPIOs in the CSF. Five weeks later, dark contrast is observed extending from the rostral tip of the lateral ventricle, diving rostrally and ventrally towards the base of the brain, and curving upwards into the OBs, where the contrast spreads out and becomes punctuated. This is precisely the RMS as indicated by the histological evaluation of the BrdU injected animals. High resolution *ex vivo* MRI detected the dark contrast diffusing into the OBs very clearly. 5 of the 24 rats injected with just particles exhibited this degree of migration. 5 of 5 animals injected with particles pre-soaked in EGF displayed robust migration. Dark contrast was not observed in the cortex, while isolated contrast spots were often observed in the bottom regions of the brain. Additionally, animals exhibited migration at different times, with robust migration observed as early as one week, and as late as five weeks.

Confocal microscopy of the SVZ showed green fluorescent particles in microglia, the macrophages of the brain, within ependymal cells, the lining of the blood-brain barrier, and within neural stem cells. Figure 2 shows a confocal microscopic image from a section that was stained for doublecortin. Chains of migrating neural precursors (red) are clearly visualized within the RMS. One precursor contained green fluorescent particles bordering the nucleus (blue). In this area, particles were also found in PSA-NCAM positive cells, indicating that these cells truly were neural precursors. Within the OB, green particles could be identified within Tuj1 positive cells, indicating that neural precursors carrying the particles could differentiate into mature neurons. Additionally, iron stained histology slides show iron particles in cells within the peri-glomerular layer.

Discussion

It is demonstrated here that neural stem cells can be labeled *in vivo* by direct injection of contrast agent into their microenvironment. The contrast agent initially labeled the stem cells and the cells lining the ventricle, but eventually was transferred to daughter cells, the neural precursors, which carried them as they migrated to the OB. While neural stem cells have been labeled in culture and transplanted to hosts (5), only direct, *in vivo* labeling allows one to study endogenous stem cell responses.. This is the first demonstration of *in vivo* stem cell labeling and endogenous stem cell tracking. As only single micron size particles are required for MRI detection, poor labeling efficiency can be tolerated and in *vivo* labeling was possible. While IHC still remains the current gold standard for determining cell type in cell migration models, cellular MRI provides the advantage of examining migratory pathways in three dimensions, and progressing over time. Lastly, due to the amplification of the MR signal due to the contrast agent, rare cellular events, difficult to observe by histology, may become apparent and new discoveries may be found.

References: 1) Shapiro EM, et al., *PNAS* 101(30), 10901-6 (2004); 2) Doetsch F, et al, *Cell* 97(6), 703-16 (1999); 3) Doetsch F, et al, *Neuron* 36(6), 1021-34 (2002); 4) Hinds KA, et al, *Blood*, **102**:3, 867-72, (2003); 5) Modo M, et al, *Neuroimage* 21(1), 311-7 (2004).

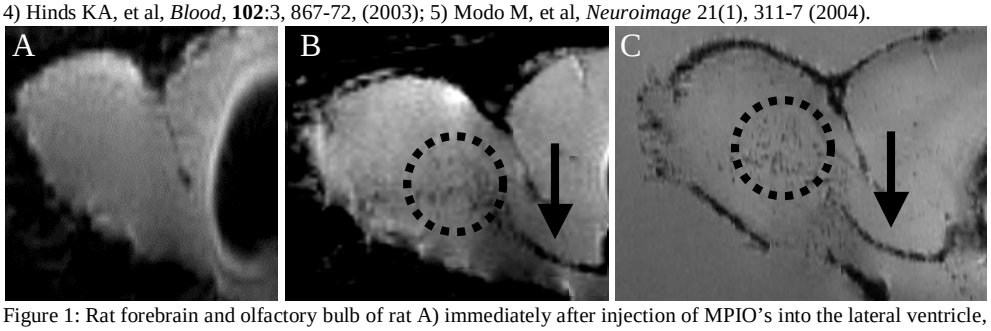

Figure 1: Rat forebrain and olfactory bulb of rat A) immediately after injection of MPIO's into the lateral ventricle, B) *in vivo* five weeks after injection, and C) *in vitro* 5 weeks after injection. Dark contrast is present in the rostral migratory stream (arrows) and in the olfactory bulb (circled).

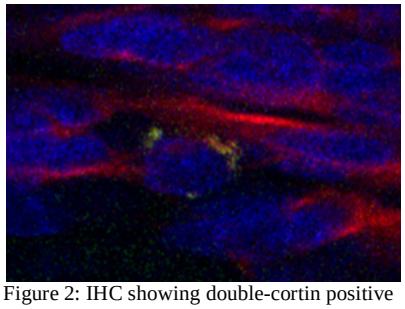

Figure 2: IHC showing double-cortin positive neural precursors (red) in the RMS. One cell also contains green fluorescent MPIOs. Nuclei are blue.