In vivo magnetic resonance imaging of ferritin-based reporter visualizes native neuroblast migration

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Introduction

The adult mammalian brain continuously generates new neurons in specific stem cell niches. Astrocytic progenitor cells at the subventricular zone (SVZ) transiently amplify and give rise to migrating neuroblasts that travel long distances along the rostral migratory stream (RMS) to reach the olfactory bulb (1). This naturally occurring process is an important model for translational studies of stem cell therapy in the brain (2). Traditional histological approaches to study adult neurogenesis only provide a two-dimensional view with a predefined orientation. Recently, a number of MRI studies demonstrated native stem cell tracking by injecting micron-sized paramagnetic particles in the mouse ventricles and labeling cells at the SVZ (3). The iron storage protein, ferritin, is becoming a widely studied 'probeless' MRI gene reporter (4,5). We recently designed a chimeric ferritin molecule fusing the L and H ferritin subunits with a polypeptide linker; this molecule (L*H) displays higher iron loading and significantly larger transverse NMR relaxation rate compared to H-rich ferritin (6). Here, we use the L*H ferritin chimera to label native primary neuronal progenitors in the mouse brain *in situ* using a viral vector and visualize their migration *in vivo* from the SVZ to the olfactory bulb. The nucleic acid-based L*H reporter approach potentially enables long-term monitoring of viable stem cells and their progeny.

Materials and Methods

All animal experiments were approved by the Carnegie Mellon Institutional Animal Care and Use Committee (IACUC). Adult female C57BL mice (Harlan, Indianapolis, IN), 5-7 weeks old, were anesthetized using intraperitoneal cocktail of ketamine and xylazine and placed in a head stereotactic device. Animals (n=20) were injected with 5 µl of type 5 adenovirus (AdV) expressing L*H under a CMV promoter ($2x10^{10}$ pfu/ml) in the SVZ area (coordinates from Bregma 1, 1, 2.2 mm). The contralateral control side was injected with GFP AdV ($1x10^{11}$ pfu/ml). Mice were imaged 10 days after injection. MRI was performed using an 11.7 T Bruker microimaging system. We acquired T_2*-weighted images using a 3D gradient-echo (GRE) sequence. For the *in vivo* imaging we used a laboratory-built surface coil with TE/TR = 7/50 ms, 4 averages, 200×200×200 image points, field of view = 1.2×1.2×1.2 cm, and 60 µm isotropic resolution. After imaging, animals were perfused transcardially with phosphate buffer saline (PBS) followed by 4% paraformaldehyde in PBS. For immunohistochemistry, we used primary antibodies that included mouse monoclonal anti-FLAG (F3165, Sigma, St. Louis, MO), rabbit polyclonal anti-neural cell adhesion molecule (AB5032, Millipore, Billerica, MA), and goat polyclonal antibody (sc-8066, Santa Cruz Biotechnology) to doublecortin (DX). Secondary antibodies were donkey anti-rabbit Alexa Fluor 488 (A-21206, Molecular Probes, Carlsbad, CA) and goat anti-mouse Alexa Fluor 594 FAB (A-11020, Molecular Probes, Carlsbad, CA). Perls' iron staining was performed on selected tissue slices.

Results and Discussion

At 10 days post-inoculation, mice were imaged *in vivo*; the MRI reporter displayed pronounced hypointensity streaming from the site of AdV-L*H injection (asterisks, Figs. 1a,b) along the RMS and towards the olfactory bulb (arrows). There was no change in contrast on the GFP control side (upper asterisk, Fig. 1b). In order to validate these findings and establish the phenotype of the migrating cells we performed series of immunohistological stains for different molecular markers. We found a considerable number of cells along the RMS region that co-stained for FLAG and PSA-NCAM (Fig. 1c). Cells in the RMS and olfactory bulb also co-stained for FLAG and DX indicating that cells labeled with the reporter have reached the bulb (data not shown). The L*H ferritin reports neuroblast migration via endogenous iron loading into its protein cage. Perls' iron stain of the tissue showed apparent iron deposits at the SVZ injection site and a large number of iron loaded (blue) cells along the RMS region (Fig. 1d). Overall, the L*H MRI reporter shows promise for non-invasive cell tracking and potentially reporting on local molecular events such as the expression of therapeutic or endogenous genes.

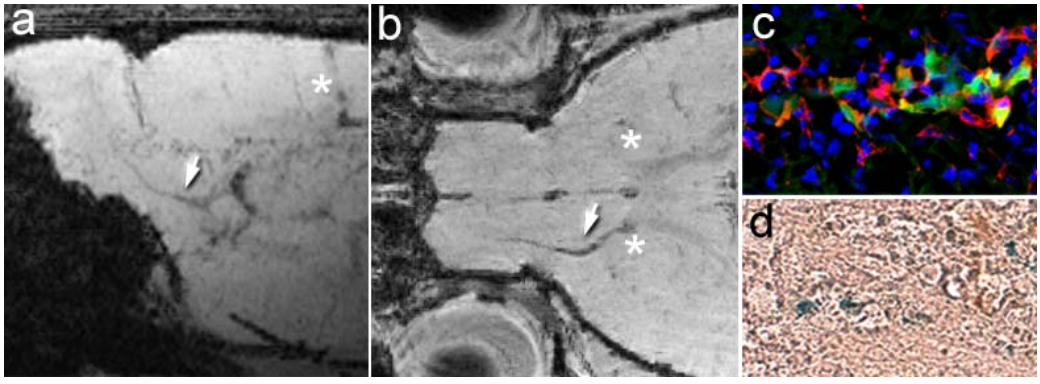

Figure 1. *In vivo* results using ferritin-based reporter to visualize native neuroblast migration. T_2* weighted MRI of saggital (a) and axial (b) projections from consecutive slices acquired 10 days post-injection of AdV-L*H in the SVZ. Arrows show a hypointense stream of cells trail along the RMS. Asterisks are AdV injection sites (GFP control is top). (c) Cells in the RMS stained for FLAG (red) in L*H construct and poly-sialated neural cell adhesion molecule, PSA-NCAM (green), a marker for migration. Cell nuclei stained with Hoechst blue. (d) Perls' iron stain shows iron rich (blue) cells at the RMS region.

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References

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