MR tracking of lineage-restricted neural precursors following transplantation into the adult spinal cord

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Introduction: Previous work has demonstrated that neuronal-restricted precursor cells (NRPs) and glial-restricted precursor cells (GRPs), whether grafted individually or together, display robust survival and extensive migration, as well as neuronal and glial differentiation following transplantation into both the intact and injured CNS. The objective of the present study was to determine if these lineage-restricted precursors can be reliably tracked by MRI following magnetic labeling and engraftment into the adult spinal cord. One of the limitations of MRI cell tracking is its use for imaging of highly proliferative cells, and few studies have addressed the impact of cell proliferation on the interpretation of the obtained MR images, as well as proper cell differentiation following magnetic labeling. Our aim was to perform a detailed study of the magnetic labeling effects as related to cell differentiation of the MR imaging validity and accuracy as compared to conventional histology.

Methods: Cells were derived from embryonic day-13.5 fetal spinal cord of transgenic donor rats that expresses the marker gene human placental alkaline phosphatase (AP) under the control of the ubiquitous Rosa 26 promoter for reliable histological tracking. Prior to transplantation, NRPs and GRPs were incubated with Feridex and poly-L-lysine for 48 hours. Labeled cells were analyzed for phenotype expression in vitro using immunohistochemistry. Sprague Dawley rats (n=9) received a single unilateral graft of 50,000 cells in 1uL into the right dorsal columns at the cervical 4 level. 5 weeks following transplantation, animals were sacrificed and spinal cord samples were imaged using a Bruker 11.7T Avance spectrometer, equipped with a 10-mm-diameter coil used for transmission and reception. 3D data sets with an isotropic resolution of 78µm (FOV: 21x50x50mm, 256x64x64 matrix) were acquired with a T2* weighted 3D gradient echo sequence (TE/TR=6.8/100, AV=4). AP histochemistry, DAB-enhanced Prussian Blue staining, and immunohistochemical surface marker staining was used for histological evaluation.

Results and Discussion: Following labeling at the time of transplantation, all cells in the cultures expressed the early neural marker nestin, confirming that the cells remained at the NPC stage. Nearly all cells in the cultures expressed either E-NCAM or A_2B_5, markers of NRPs and GRPs, respectively, confirming that they were composed almost exclusively of lineage-restricted precursors. On the MRI's, transplanted cells were detected near the injection site in both the white and gray matter; however, migration of engrafted cells was noted selectively along white matter tracts (dorsal columns) of the intact spinal cord to distances up to 5mm from the engraftment site (**Fig. 1A-C**). Following ex vivo MR imaging of spinal cords, imaged tissue was examined by histology to correlate Prussian Blue and AP detection of grafted cells with MRI results. PB staining coincided with MR images (**Fig. 1D**), demonstrating that MRI accurately detects the location of iron oxide-containing cells cells. The Prussian Blue staining demonstrated that grafted cells retained iron oxide particles in vivo. Overall, AP histochemistry correlated with the MRI and PB distribution pattern, except for an occasional cell mass containing rapidly dividing cells (**Fig. 1E**, asterisks). AP-positive and Prussian Blue-positive cells were found in both white and gray matter, and these cells were also found at distances up to 5mm from the transplantation site, in accordance with the MR images. Transplanted, labeled cells differentiated normally into all 3 mature cells types of the adult CNS: neurons (**Fig. 2A**-AP**, 2B**-NeuN**, 2C**-overlay), oligodendrocytes (**Fig. 2D**-AP**, 2E**-RIP**, 2F**-overlay) and astrocytes (**Fig. 2G**-AP**, 2H**-GFAP**, 2I**-overlay). Finally, transplant recipient spinal cords were analyzed for the presence and location of cells of the monocytic/microglia lineage. Double immunofluorescence staining for AP (**Fig. 3A**) with ED1 (**Fig. 3B**) showed the spatial relationship of host macrophages/microglia to transplanted iron-oxide labeled cells (**Fig. 3C**). Feridex-labeled cells promoted the infiltration of ED1-positive cells to the site of engraftment. However, the location of ED1-positive cells did not overlap with the location of AP labeled or Prussian Blue labeled (**Fig. 3C**) cells in graft-recipient spinal cords, suggesting that the MRI analysis detected the presence of transplanted cells, but not macrophages/microglia that had taken up the label from grafted cells. Double immunofluorescence staining for AP (**Fig. 3D**) with ED1 (**Fig. 3E**) showed the spatial relationship of host macrophages/microglia to transplanted cells that were not labeled with iron oxide. Unlike iron oxide labeled grafts, almost no ED1-positive cells were located at the site of injection following transplantation of unlabeled NRPs/GRPs.

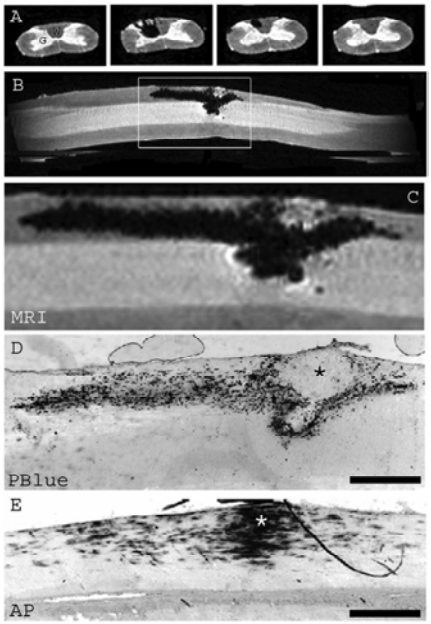

Figure 1

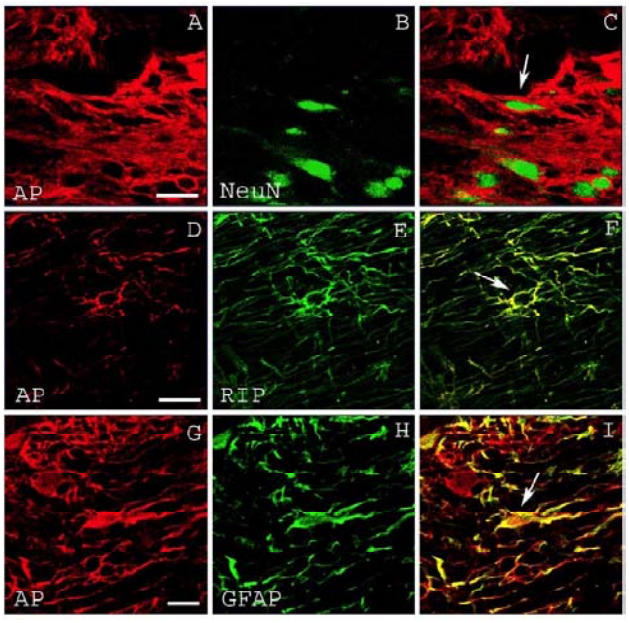

Figure 2

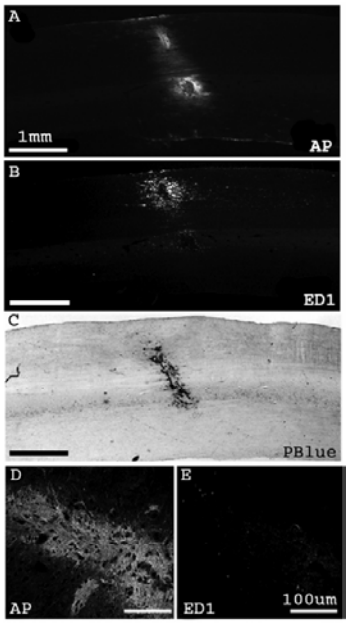

Figure 3

Conclusions: Iron oxide labeled NRPs and GRPs, without losing their normal robust fate *in vivo*, can be reliably tracked with MRI for extended periods of time following transplantation into the adult CNS. These results are important for the clinical application of NPC transplantation strategies in the treatment of spinal cord injury and other CNS pathologies. In addition, they further highlight the utility of transplanting lineage-restricted NPCs derived from the embryonic CNS into the traumatically injured and degenerating nervous system.