Complete Clearance of Iron Oxide from Intracerebrally Transplanted, Proliferating Neural Stem Cells

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Introduction:
Cell-based therapy for neurodegenerative diseases is actively pursued in preclinical models and has considerable potential for clinical translation. However, optimization of such therapies to achieve clinical improvement justifying clinical translation is needed and can be expedited by the use of non-invasive cellular imaging. While MR imaging of magnetically labeled cells is considered the method of choice for cellular imaging, there are several limitations that need to be considered. Two primary issues are proliferation of labeled cells leading to dilution of the label and cell death with potential transfer of contrast to surrounding endogenous cells. Stem cell transplantation is unavoidably associated with cell turnover (proliferation and cell death) thus for accuracy of cellular imaging it is critical to address these issues. The purpose of this study was to determine the fate of superparamagnetic iron oxide nanoparticles at very late time points following the transplantation of labeled cells. To this end, we transplanted Feridex-labeled, bioluminescent neural precursor cells into the brain of immunodeficient mice and followed their survival/proliferation and persistence of iron-related hypointensity in MRI for a period of 95 days.

Methods: Mouse neural stem cells (C17.2) were transduced with the lentiviral vector pLenti4-CMV-fLuc2 in order to express the bioluminescent reporter gene firefly luciferase. Transduced cells were expanded and further labeled with the MR contrast agent Feridex (Berlex). Labeled cells ($3x10^5$/3µl) were stereotaxically implanted into corpus callosum of immunodeficient Rag2 mice. BLI imaging was performed at various time points from 0 to 95 days after cell implantation. For BLI, mice were anesthetized with isoflurane and imaged using an IVIS 200 system (Xenogen). *In vivo* MRI was performed on a Bruker 9.4T spectrometer at day 1 and day 95 after cell implantation with a T2-weighted spin echo sequence (TE/TR=26/2000ms, AV=2, RES=230x156µm). For validation of imaging results, brain tissue was cryosectioned and stained with Prussian blue for detection of Feridex iron. Immunohistochemical detection of transplanted cells was performed using anti b-galactosidase antibody (Cappel).

Results: Implanted magnetically labeled, luciferase-expressing C17.2 cells generated detectable BLI signal (Figures **A,B**) on the day of transplantation. During a period of about 60 days, the BLI signal was gradually increasing to a plateau at the level of 1000-fold that of the initial signal (Graph **A**). Ninety-five days after transplantation, a strong BLI signal was detected originating from the entire brain area (Figure **D**), while the MRI hypointensities became undetectable (Figure **E**). Histology revealed that b-gal positive transplanted cells were detected in large numbers with quite limited migration, occupying a limited area in the proximity of the injection site (Figure **F**). Prussian Blue staining showed only few positive cells mostly within white matter tracks (Figure **G**).

Conclusions: Transplantation of magnetically labeled, proliferating cells into mouse brain leads to dilution of iron oxide label with complete clearance of contrast agent over a period of 95 days. Lack of residual contrast indicates that a potential transfer of label to local tissue macrophages is followed by complete biodegradation of superparamagnetic iron oxide.

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