MRI of Magnetically Labeled Endothelial Precursor Cells to Non-Invasively Image Neovasculature in a Mouse Glioma Model

S. A. Anderson[1], J. Glod[2], A. S. Arbab[3], M. Noel[4], P. Ashari[3], H. A. Fine[4], J. A. Frank[3]

[1]NINDS, National Institutes of Health (NIH), Bethesda, MD, United States, [2]Pediatric Hematology/Oncology, Cancer Institute of New Jersey, New Brunswick, NJ, United States, [3]ENS/LDRR, NIH, Bethesda, MD, United States, [4]NCI and NINDS, NIH, Bethesda, MD, United States

Background: Bone marrow-derived endothelial precursor cells (EPCs) incorporate into tumor neovasculature and have been successfully used as vehicles for gene delivery to tumors (1). *In vitro* labeling of EPCs with SPIO nanoparticles has the potential to directly detect the migration of these cells toward angiogenic stimuli and incorporation within developing tumor neovasculature by MRI. The purpose of this study was to determine if MRI would detect the incorporation of FE-PLL labeled Sca1+ EPCs into neovasculature in a mouse glioma model and to differentiate results from animals receiving unlabeled cells.

Methods*: Animal model:* Experiments were performed in accordance with NIH animal care and use guidelines. $5x10^5$ RT2 rat glioma cells were transplanted by intracranial injection into 5-week old SCID mice. Sca1+ cells were isolated from the marrow of Swiss mice using anti-Sca1 magnetic beads (Miltenyi Biotec, Germany). Cells were incubated with a complex of SPIO nanoparticles (Ferumoxides, Berlex Laboratories, Wayne, NJ) and Poly-L-lysine (388 kD, Sigma, St.Louis, MO) (FE-PLL), using procedures previously described (2,3,4). Unlabeled or killed labeled EPCs were used as controls. Mice received 5×10^5 labeled or control cells via tail vein usually two days after tumor implant. Twelve mice received labeled EPCs, 4 unlabeled, and 2 killed labeled cells. Labeled and unlabeled mice had a similar clinical course. *Magnetic resonance imaging:* Mice were imaged using a 7T 22 cm horizontal bore magnet (Bruker, Billerica, Mass) with 39G/cm gradients and a 35 mm birdcage volume coil. Mice were anesthetized with isofluorane and imaged using 2D gradient echoes (GE) (TR/TE= 400/4.0 ms), 2D spin echoes (TR/TE=2200/7.8 ms; 3 echoes), or 3D RARE images (TR/TE=1300/7.1 ms; rare factor 8). Resolution was ca. 500x80x70 □m in 2D, 93 to 117 □m isotropic in 3D. Post-contrast-enhanced 2D GE images were obtained 20 minutes following *sq* injection of 0.4 mmol/kg GdDTPA(Magnevist, Berlex Laboratories, Wayne NJ) in 2 mice. Mice were euthanized, perfused with 4% paraformaldehyde, and fixed brains were imaged in a 7T microimaging system (Bruker), with 3D GE (TR/TE=300/5.7 ms), 45-50 micron isotropic resolution. *Histopathology*: Histopathology was performed on 10 μm slides from paraformaldehyde-fixed brains. Prussian blue for iron and CD31 staining were performed using standard methods. ***Results****:* MRI of mice with labeled cells demonstrated hypointense regions within the tumor, which evolved over time into a continuous hypointense ring. This effect was not seen on mice receiving unlabeled cells (**Fig. 1a,b**) and was not cleared by administration of Gd-DTPA. Histopathology showed iron-labeled cells around the tumor rim in labeled mice (**Fig. 1c**). Morphologically these cells appeared elongated and in close proximity to vessels, and a large proportion of iron-labeled cells were positive for an endothelial cell marker, CD 31. ***Conclusions****:* MRI demonstrated the presence of labeled cells within and surrounding the tumor. Histopathology showed iron labeled cells in similar distribution in brain, and indicates these cells have differentiated into endothelial-like cells. These results demonstrate that MRI can be used to noninvasively monitor the incorporation of magnetically labeled EPCs as part of ongoing angiogenesis providing a means to directly identify neovasculature as well as to track and optimize delivery of these cells as gene delivery vectors *in vivo*.

References*: 1. Ferrari N et al. GeneTherapy 2003;10:6471 2. Frank JA ,et al. Acad Rad 2002;9:S484. 3. Frank JA et al. Radiology 2003;228:480, 4. Arbab AS et al Transplantation 2003;76:1123*

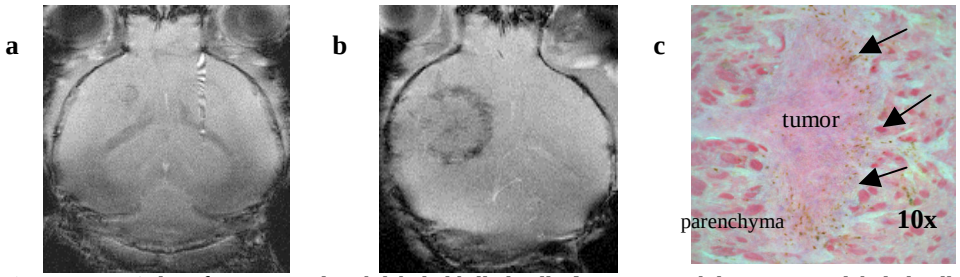

Fig.1. *In vivo* MRI at 10 days for **a**. control with labeled killed cells, **b**. mouse with live FE-PLL labeled cells. Control has minimal darkening effects in the tumor or periphery compared to the mouse receiving labeled cells. **c**. DAB-enhanced Prussian blue stain of fixed brain. Iron-labeled cells (stained brown) are clustered around the tumor periphery.